The role of fast-spiking interneurons in cortical map plasticity

Albieri, Giorgia

Awarding institution:
King's College London

THE ROLE OF FAST-SPIKING INTERNEURONS IN CORTICAL MAP PLASTICITY

Giorgia Albieri

INSTITUTE OF PSYCHIATRY
KING'S COLLEGE LONDON

Submitted for the PhD in Neuroscience

Abstract

Rodents have a topographic map in primary somatosensory cortex of the contralateral facial whiskers. A brief period of whisker trimming causes the representation of the non-trimmed whiskers (spared) to expand into the cortex that has lost its principal sensory input (deprived). It has been hypothesized that this is mediated by a period of persistent disinhibition in deprived cortex that enables the expansion of spared whisker representations. Alternatively, it has been proposed that inhibition undergoes a biphasic change with an initial, brief period of disinhibition to promote plasticity in excitatory circuits followed by a more prolonged increase in inhibition to re-establish the excitatory – inhibitory balance. These hypotheses make different predictions about how inhibition changes during cortical map plasticity, which I have tested in this thesis.

I focused on fast-spiking (FS) interneurons, which are thought to play an important role in adult cortical plasticity. I made electrophysiological recordings in layer 2/3 to determine how inhibitory circuitry in deprived cortex is affected by whisker deprivation. The amplitude of miniature excitatory postsynaptic potentials (mEPSPs) in deprived FS interneurons was increased with no change in mEPSP frequency suggesting that the global excitatory drive onto FS interneurons was potentiated. In contrast, the amplitude of miniature inhibitory postsynaptic currents (mIPSCs) in layer 2/3 pyramidal neurons was unchanged, but there was a small but significant increase in mIPSC frequency. I investigated feedback inhibitory circuits in more detail by recording from pairs of pyramidal cells and FS interneurons that were synaptically-connected. Surprisingly, I found that the strength of local excitation onto FS interneurons and the strength of FS – mediated inhibition on deprived pyramidal neurons were unchanged. I concluded that, contrary to two popular hypotheses, a brief period of sensory deprivation did not alter the feedback inhibition in layer 2/3 of deprived cortex.

Table of Contents

Table of Figures

Table of Tables

Acknowledgements

The time I spent working on this project has been extremely enjoyable, because of the immense support of co-workers, family and friends.

Foremost, I would like to express my sincere gratitude to my supervisor, Dr. Gerald Finnerty, for giving me the opportunity to join his lab. His mentorship and guidance have helped me to grow, as a scientist and as a person. He believed in me more than I believed in myself, and for this I will always be grateful. He also wrote the programs for the electrophysiology analysis.

Un grazie speciale alla mia mamma e al mio papa`, che sono i genitori migliori del mondo. Senza il loro appoggio e il loro affetto (e ogni tanto la loro Mastercard) non ce l'avrei mai potuta fare.

The time I spent in the lab has been great fun, mainly because of the wonderful people I had the fortune to work with. Dr. Sam Barnes has been a fantastic teacher and a great science-husband. His support, enthusiasm and love for science (and beers) made the long hours in the lab manageable. Dr. Claire Cheetham has also been extremely helpful, despite we worked together only a few months. Her passion and determination helped me to find the strength to persist and succeed. More recently Catherine Smith has shared with me the joy and pain of electrophysiology and the taste for fashion clothes. Sui Poh and Liu Yan have also been very supportive, feeding me with Chinese delicacies and sweets.

Least, but not last, the two persons that know me best and supported me most: Alice and Matthew. My sister Alice was always on my side, despite the kilometres that divide us (and our divergences in matter of art and politics).

Matthew, there are no words to describe how important you are for me and how helpful and supportive you have been during my PhD (as if your PhD was not enough). I can only say thanks to be part of my life.

Abbreviations

ACSF	Artificial cerebrospinal fluid
AF	Alex Fluor (dye)
AHP	Afterhyperpolarisation
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
AP	Action potential
AU	Airy unit
BDNF	Brain derived neurotrophic factor
BOLD-fMRI	Blood oxygen level-dependent functional magnetic resonance imaging
BSNP	Burst spiking non-pyramidal
Cm	Membrane capacitance
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
CSPG	Chondroitin sulphate proteoglycans
APV	D-Aminophosphonovaleric acid
EPSC	Excitatory postsynaptic current
EPSP	Excitatory postsynaptic potential
fAHP	Fast afterhyperpolarisation
FS	Fast-spiking interneuron
GABA	Gamma-aminobutyric acid
GAD	Glutamic acid decarboxylase
GFP	Green fluorescent protein
HFT	Dichroic filter
Hz	Hertz
IPSC	Inhibitory postsynaptic current
IPSP	Inhibitory postsynaptic potential
IQR	Interquartile range
IR-DIC	Infra-red differential interference contrast
ISI	Interspike interval
KCC2	Potassium chloride co-transporter
kHz	Kilohertz
LTD	Long-term depression

LTP	Long term potentiation
MD	Monocular deprivation
mEPSC	Miniature excitatory postsynaptic current
mEPSP	Miniature excitatory postsynaptic potential
mIPSC	Miniature inhibitory postsynaptic current
mM	Milimolar
mOsm	Miliosmoles
ms	Milisecond
mV	Milivolt
mW	Miliwatt
MΩ	Megaohm
nA	Nanoamps
NFT	Secondary dichroic
nm	Nanometre
NMDA	N-methyl D-aspartate
NFS	Non fast-spiking interneuron
OD	Ocular dominance
P	Postnatal day
p =/<	Probability equals/ less than
pA	Picaamps
PBS	Phosphate buffer solution
PFA	Paraformaldehyde
PSC	Postsynaptic current
PSP	Postsynaptic potential
PTX	Picrotoxin
PV+	Parvalbumin-positive
Pyr.	Pyramidal neuron
QX314	Lidocaine bromide (N-2,6-dimethylphenylcarbamoylmethyl triethylammonium bromide)
Rm	Membrane resistance
RS	Regular spiking
SEM	Standard error
sIPSC	Spontaneous inhibitory postsynaptic current

STDP	Spike-timing dependent plasticity
TTX	Tetrodotoxin
uEPSP	Unitary excitatory postsynaptic potential
uIPSP	Unitary inhibitory postsynaptic potential
Vm	Membrane potential
VPM	Ventroposteriomedial nucleus
µg	Microgram
µM	Micromolar
µm	Micrometres
µs	Microsecond
τm	Membrane time constant

Chapter 1 Introduction

1.1 Sensory representations in the brain

A discrete portion of neocortex receives and processes information coming from the sensory periphery. The peripheral input representations are organized topographically, with different areas assigned to specific sensory systems. This organization generates cortical maps of sensory representations in the neocortex (Buonomano and Merzenich 1998). Interestingly, there are multiple cortical maps for each representation within the sensory cortex (Kaas 1987, Alonso et al. 2008) each of those is likely to be involved in different stages of information processing. However, the most extensively studied representations are those located within the primary sensory cortex (SI).

It is now widely recognized that sensory maps, although established and defined during a critical period of development, are not fixed entities but they are rather dynamically regulated by sensory experience, even in adulthood (Buonomano and Merzenich 1998). The pioneering studies in primates by Merzenich *et al*. showed that sensory deprivation by transection of the median nerve in the monkey alters the corresponding sensory map located in the cytoarchitectural areas 3b and 1 (Merzenich et al. 1983b, Merzenich et al. 1983a). The following immediate loss of responsiveness in these areas is however not permanent. The cellular circuits in the corresponding areas undergo a reorganization process, which results in the expansion of the surrounding representations into the cortex previously associated with the median nerve (Merzenich et al. 1983a). Since then, cortical maps reorganization has been studied in many different sensory systems and species (Buonomano and Merzenich 1998), and magnetoencephalographic (MEG) and electroencephalographic (EEG) mapping studies have suggested that similar reorganization processes also occur in the human brain following limb amputation (Elbert et al. 1994).

All these experiments proved that traumatic lesions of the sensory periphery result in cortical map plasticity. However, non traumatic sensory deprivation can also result in

cortical reorganization (Simons and Land 1987). Moreover, sensory deprivation is not the only process that alters cortical representations. Training in a frequency discrimination task in adult monkeys leads to expansion of the correspondent cortical representation (Recanzone et al. 1992b, Recanzone et al. 1992a). All together, cortical map reorganization reflects one of the most intriguing properties of the brain; its ability to adapt in response to changes in the individual's environment. Despite the many efforts made in the last two decades to understand the cellular mechanisms underpinning cortical map reorganization, the process is still incompletely understood.

1.2 Why is it important to study experience-dependent plasticity?

Understanding the mechanisms by which the brain operates and adapts to the external environment is still one of the biggest biological challenges. Studying the basic plasticity mechanisms that occur in the adult brain is also vital to improve the recovery from cortical damage (Fox 2009). Plasticity is in fact implicated in recovery from a vast group of diseases, including neurodegenerative conditions (Palop et al. 2006) and stroke (Nudo 2006). Increased plasticity is however not sufficient for recovery from injury, but it requires a contribution from sensory information to selectively target specific connections (Fox 2009). Therefore, experience – dependent plasticity in adulthood potentially not only provides the mechanisms for functional recovery from injury, but also for learning and memory (Hubener and Bonhoeffer 2010).

In this thesis I will investigate the plasticity mechanisms by which cortical circuits reorganize when sensory experience is altered. Neural circuits consist of connections between neurons, which can be either excitatory or inhibitory (Peters and Jones 1984). The balance between excitation and inhibition is fundamental for the stability of neural circuits (Galarreta and Hestrin 1998, Hensch and Fagiolini 2005, Yizhar et al. 2011). Therefore, I will focus my attention on the interaction between excitatory and inhibitory

neurons. I will conduct my investigations in the barrel cortex of rats, which is part of their somatosensory system. As it is widely recognised, plasticity mechanisms are shared between cortical areas (Fox and Wong 2005) and different species (Fox 2008). I hope my research, by improving our understanding of plasticity in cortical map reorganization, will be applicable in a broad range of brain areas and conditions. Based on the same principle, I will report recent findings from other sensory cortices or hippocampus when relevant to the topic of discussion.

In Section 1.3.1, I will describe the barrel cortex and the anatomical pathways of the whisker system, which are important to set my research into context. I will then describe the main characteristics of the cortical neuron populations, with major focus on inhibitory neurons (Section 1.3.2). In Section 1.4 I will summarise our actual knowledge on developmental and adult plasticity in barrel cortex, and I will report the evidence for the contribution of inhibitory circuits to cortical plasticity. The cellular mechanisms of inhibitory circuits' plasticity and the experience – dependent plasticity of inhibitory circuits are described in Section 1.5. I will finally conclude setting the rationale for the aim of this study (Section 1.7).

1.3 The barrel cortex

Rodents have developed a peculiar tactile perception system that consists of specialized hair located on both sides of their snout, called vibrissae or whiskers. The barrel cortex is the portion of primary somatosensory cortex that receives and processes information coming from whiskers. This sense is extremely well developed. On its own, it represents almost 70% of the whole somatosensory system in rodents (Fox 2008). The whiskers usually move during explorative behaviours (Moore et al. 1999) and can detect not just edges and surfaces, but also perform textures discriminations (Diamond et al. 2008a).

There are at least three main reasons why the barrel cortex is an optimal system to investigate the cellular mechanisms of cortical map reorganization. Firstly, the thalamic afferents carrying information from the whiskers project principally onto the layer 4 of the primary somatosensory cortex, where they form clear cell aggregates called barrels. The barrels can be easily visualized using different staining methods, such as cytocrome oxidase (Land and Simons 1985) and Nissl stain (Woolsey and Van der Loos 1970), but they are also visible under transillumination microscopy in living brain slices (Finnerty et al. 1999). Secondly, the spatial relation between the barrels in the primary somatosensory cortex of the contralateral hemisphere coincides with the topological organization of the whiskers in the snout of the animal. These two properties together give us the big advantage of being able to visualize the barrels, knowing which one corresponds to each specific whisker. Finally, sensory experience can be altered in this system by non traumatic whisker trimming. This procedure is very simple, innocuous for the animals and it induces cortical map reorganization (Fox 1992, Simons and Land 1987).

1.3.1 The whisker system

1.3.1.1 Anatomical pathways

The rodents' whiskers are specialized hairs that work as proper tactile organs. The vibrissae are located on the snout of the rats in five rows (A-E, dorsal to ventral) of four to six whiskers each (1 - 4 arcs, caudal to rostral) plus four more caudal longer, straddler whiskers (α, β, γ and δ) sited between the rows (Welker and Woolsey 1974).

The whisker grows from a skin cavity called whisker follicle that is innervated by motor nerves to control its movement and by sensory nerves, which carry specific signals for each whisker. Follicle receptors can be both slowly or rapidly adaptating, to collect information about type, amplitude and speed of the whisker deflection (Fox 2008). Each whisker is innervated by two different types of nerve: a vibrissae nerve and a deep vibrissae nerve, which both contain about 200 axonal fibers (Fox 2008). The cellular bodies of these sensory neurons are located in the trigeminal ganglion. Their axons form

part of the infraorbital nerve, which subsequently merges with other branches of the fifth cranial nerve to transmit information to the trigeminal nuclei of the brainstem. All four trigeminal nuclei, named principalis, oralis, caudalis and interpolaris, have somatotopic whisker representations which are referred as barrellettes (Chiaia et al. 1992). The cells in the barrellettes are activated only by the deflection of the specific whisker to which they are associated. However, the vast majority of afferents from the brainstem to the thalamic nuclei come from the nuclei principalis and interpolaris, from which three distinct information pathways are generated.

The vast majority of cells in the trigeminal nucleus principalis project only to the ventro-posterior medial nucleus of the thalamus (VPM) (Chiaia et al. 1991). In the thalamus, the somatotopic representations of the whiskers are also discernible, and they are referred as barrelloids (Diamond et al. 1992, Petersen 2007). Each barelloid is still activated by the deflection of the corresponding vibrissae. However, weak activations also occur by the deflection of surrounding whiskers (Fox 2008). The axons of VPM cells terminate mainly on the layer 4 of the primary somatosensory cortex, where they create the barrels, with a minor portion ending on layer 5B, layer 6 and a few cells in layer 3 (Woolsey and Van der Loos 1970, Brecht and Sakmann 2002). The stream of sensory information that goes from the mechanoreceptors of the sensory cells to the trigeminal nucleus principalis of the brainstem and in turn to the barrels via the VPM thalamic nucleus is called the leminiscal pathway (Fox 2008, Petersen 2007).

A large portion of axons from the caudal sides of the barrelletes in the interpolaris trigeminal nucleus also project to the thalamic VPM, but in a specific ventrolateral strip which in turn project to the septa, the regions of layer 4 primary somatosensory cortex interposed between the barrels, and the secondary somatosensory cortex. This pathway is referred as extralemniscal pathway (Diamond et al. 2008b).

Finally, the paralemniscal pathway is generated by a minor fraction of rostral afferents from the interpolaris nucleus that reaches the posterior medial thalamic nucleus (POM) (Fox 2008). These thalamic afferents contact all the layers of the barrel cortex, but predominantly layer 5 and other cortical areas, such as the secondary somatosensory cortex and the motor cortex (Fox 2008).

The existence of multiple pathways suggests that they convey different types of information. Specifically, the paralemniscal, extralemniscal and lemniscal pathways would transmit information about kinematics, time of contact and touch respectively (Diamond et al. 2008b). However, these functional subdivisions are still debated (Diamond et al. 2008b).

1.3.1.2 The flux of information within the barrel cortex

The neocortex presents a functional organization in vertical modules called columns, which are thought to be the functional units of information processing (Mountcastle 1997). The barrel cortex has the big advantage of making the columnar organization clearly visible, since each barrel identifies the column associated with the corresponding whisker. Following a whisker deflection, the signal, which starts in the sensory neurons and passes through the brainstem nuclei to the thalamus, reaches the barrel cortex. The main cortical recipient of thalamic projections from VPM is layer 4, which is the starting point of an intracolumnal flux of information that moves across different layers of the neocortex (Pierret et al. 2000). Despite thalamocortical projections being the primary carrier of sensory information, they account only for 15% of the overall number of synapses made on dendrtic arborizations of spiny excitatory cells of layer 4 (Benshalom and White 1986), whereas the vast majority of excitatory synapses reflect intracortical connections within layer 4 (Feldmeyer 2012). Moreover, the efficacy of the thalamcortical synapses is rather weak, so that multiple convergent and synchronous inputs are required to fire the layer 4 excitatory cells (Bruno and Sakmann 2006). Layer 4 excitatory cells have arborisations that rarely extend outside the boundaries of the column and are connected between each

other into the same barrel, amplifying the excitation coming from the thalamus (Feldmeyer et al. 1999). Inhibitory cells in layer 4 also receive thalamic inputs (Bruno and Simons 2002), which play a fundamental role in feedforward inhibition.

The wave of excitation that extends from the thalamus to layer 4 reaches the pyramidal cells of layer 2/3 directly in the same column (Feldmeyer et al. 2002), as it has been also shown by voltage sensitive dye imaging (Petersen and Sakmann 2001). Layer 2/3 cells also respond to input coming from neighbouring barrels, which is mainly mediated by long distance horizontal connections between excitatory cells within layer 2/3 (Fox 2008). Inhibition is also extremely important in layer 2/3, with both feedforward inhibition from layer 4 and feedback inhibition in layer 2/3 acting to suppress the potentially epileptogenic spread of excitation. The roles and organization of inhibitory circuits will be further addressed in the following sections. Layer 2/3 excitatory cells also project to layer 5 cells (Reyes and Sakmann 1999), which receive various inputs both from other cortical layers and the thalamus, to integrate the information before transferring it to other sub cortical regions. The “layer 4 → layer 2/3 →layer 5” microcircuit seems to constitute the main information pathway and it is definitely the most studied in the barrel cortex. However, it is very likely that both the para-lemniscal and extra-lemniscal pathways are also strongly involved in information processing, although their precise role has not yet been completely elucidated (Diamond et al. 2008b).

1.3.1.3 Cortical output

The flow of information does not stop with the processing across different layers of the primary somatosensory cortex. In fact, different cortical layers project both to other non primary cortical areas and subcortical structures (Fox 2008). Barrels in the same rows project to single strips in secondary somatosensory cortex (SII), mainly from layer 3, 6 and 5 (Chakrabarti and Alloway 2006). The projection to primary motor cortex (MI) comes primarily from septal cells (Alloway et al. 2004), some of which also connect to SII.

The subcortical projections are directed primarily to other motor structures or back to the thalamus. Layer 5b cells project to the pons and to the superior colliculus, while layers 5A, a small fraction of layer 5B and the layer 5 cells located in the septa project to the striatum (Fox 2008). Layer 5 and 6 cells also project back to the VPM nucleus, where the topographic organization is maintained, as cortical afferents end back in the corresponding barrelloids (Wright et al. 2000). Similarly, cells from layer 5 septa project back to the VPO (Wright et al. 2000). Finally layer 5 cells also project back to the trigeminal nuclei (Fox 2008).

1.3.1.4 Barrel cortex summary

To summarize, the deflection of a whisker on the animal snout generates a stream of information that flows from the sensory cells in the trigeminal nuclei to the thalamus and from there to the layer 4 of the barrel cortex. The cells in layer 4, in turn, project to layer 2/3, where the activity spreads outside the limit of the column, possibly as part of an integration process. It has been argued that the role of layer 2/3 is to integrate different inputs from different whiskers, mainly because layer 2-3 pyramidal cells are connected locally, but also laterally (with neurons in surrounding barrel columns) within the same layer (Feldmeyer 2012). Moreover, the activation of cells in layer 2/3 leads the signal to spread vertically to layer 5, the main output layer. The projections from cortex to other different brain areas are beyond the aim of this project. It is however important to point out their existence, because although cortical plasticity will be investigated here only in primary somatosensory cortex, evidence suggests that map in secondary somatosensory cortex and in the parietal ventral area are also affected by sensory deprivation (Alonso et al. 2008).

1.3.2 From cells to circuits

In the previous section, the anatomical pathways that convey information from the whiskers to the cortex have been extensively described. In the prospective of cortical map reorganization, it is important to point out that the cortical cell population is composed of many different excitatory and inhibitory cell types. To investigate the cellular and molecular mechanisms behind cortical map plasticity, it is fundamental to identify which circuits of the neocortex are altered by sensory experience. Ultimately, a neuronal circuit can be defined as a set of connections between neurons. Therefore, in studying inhibitory or excitatory circuits, it is important to consider: the characteristics of the cell types that are part of the circuit; and the cellular mechanisms by which the output of the circuit and configuration can be altered.

1.3.2.1 The cellular population of the neocortex

The necortical neurons can be broadly classified in two major classes: excitatory cells and inhibitory cells (Peters and Jones 1984). Excitatory cells are the more abundant class. They release glutamate and have extensively developed dendritic and axonal arborisations. On contrast, inhibitory cells account for about 20 – 30% of neocortical neurons (Markram et al. 2004). They use GABA as neurotransmitter and don't project outside the cortex, but they play a crucial role within local circuits (Markram et al. 2004, Ascoli et al. 2008).

1.3.2.1.1 Excitatory cells

In layer 4, there are two main types of excitatory cells: spine stellate cells and star pyramids (Lubke et al. 2000). Spiny stellate cells have spherical to ovidal soma, asymmetric dendrites that are confined within the barrel column and axon collaterals that extend into all cortical layers (Lubke et al. 2000). Star pyramids are less abundant than stellate cells; they have symmetric dendritic arbors and ovoidal somata. The excitatory cells of the other layers are generally referred as pyramidal cells, because of the pyramidal soma shape, with a prominent apical dendrite extending towards the pial surface.

However, morphological and electrophysiological characteristics can also vary between pyramidal cells, from layer to layer or even within the same layer. The pyramidal cells of layer 2/3 are easy to identify because of their soma shape and their apical dendrite, which is longer than in other cells types and can be easily distinguished from the other dendrites (Feldmeyer et al. 2006). Their axons project in layer 2/3, layer 5 and layer 1, with some collaterals reaching the white matter and other cortical areas (SII) (Feldmeyer et al. 2006). Pyramidal cells of layer 5 can be classified into two subtypes: tufted regular spiking pyramidal cells, and intrinsic bursting pyramidal cells (Fox 2008).

1.3.2.1.2 Inhibitory cells

Inhibitory interneurons represent about 20 - 30% of the neocortical cell population (Markram et al. 2004). Morphologically, they differ from pyramidal cells because: they have ovoidal or spheric somata, their dendrites are rarely spiny, but mainly completely aspiny, and their axons project predominantly locally and never outside of the neocortex (Markram et al. 2004). Interneurons are a very heterogeneous class of cells that can be classified based on: morphological properties, molecular marker expression and electrophysiological behaviour (Kawaguchi 1995, Kawaguchi and Kubota 1997, Markram et al. 2004, Ascoli et al. 2008). Despite many efforts to reach a global classification, the classes identified by the previously mentioned systems do not overlap completely. Moreover, such different characteristics, especially in firing properties, are likely to reflect different functional roles, which have not yet been identified.

1.3.2.1.2.1 Morphological properties

Interneurons can be classified based on their morphological properties, such as soma shape, dendritic arborisation, target cells and type and abundance of connections (Ascoli et al. 2008). Based on those, the following classes have been identified:

- Basket cells: they owe their name to the basket like shape of the axonal arborisation that surrounds their target cells, where the vast majority of contacts are somatic or located on the proximal dendrites. They represent almost half of the whole GABAergic neocortical population and can be further sub-classified in large, small and Nest basket cells, based on their soma dimension and extent of axonal and dendritic arborisations. Basket cells are the largest subtype of interneurons in layer 2/3 and they are thought to be the principal source of inhibition within local circuits (Gentet 2012).

- Chandelier cells: they are multipolar or bitufted cells and they own their name to their particular axonal terminals, where the boutons are disposed in short vertical rows (Somogyi et al. 1982). They are particularly abundant at the border between layer 1 and layer 2/3 (Woodruff et al. 2009). These terminals innervate the axonal initial segment of pyramidal cells, and are therefore very likely to strongly affect the generation of action potentials in the postsynaptic cell. Because of their specific innervations onto the axonic initial segment of target cells they are also known as “axo-axonic cells”.

- Martinotti cells: they have ovoidal soma with bi-tufted dendrites (clusters of dendrites protruding from two opposite poles), whose arborisation is the most extensive of all interneurons, although it is maintained within a column diameter. Their axons project to layer 1, where they can make synaptic contacts mainly onto the dendritic shafts of pyramidal cells in neighbouring or even distant columns.

- Bipolar cells: they have small spherical-ovoidal somata with bipolar (two single dendrites protruding from two opposite ends) or bi-tufted dendrites that develop in a narrow strip both in layer 1 and in layer 4.

- Bitufted cells: they are characterised by bi-tufted dendritic arborisations vertically orientated and have axons that span widely horizontally.

- Neurogliaform cells: they have small round soma with multipolar dendrites that develop radially to generate a spherical arborisation. Their axons have many collaterals that contact postsynaptic cells on dendrites. Of note is the fact that these cells make electrical synapses with each other and, less frequently, also with other interneuron types as basket cells and chandelier cells (Simon et al. 2005).

1.3.2.1.2.2 Molecular properties

Inhibitory neurons have been also classified by the pattern of expression of calcium binding proteins (CBP) and neuropeptides (Cauli et al. 1997, Kawaguchi and Shindou 1998, Markram et al. 2004, Somogyi and Klausberger 2005, Yuste 2005, Ascoli et al. 2008). Interneurons can express three different types of CBP: parvalbumin (PV), calbindin (CB) and calretinin (CR) that function as internal calcium buffers. These proteins tend to be expressed in different interneuron populations, although there is some overlap between PV and CB expression (Kubota et al. 1994). In addition to the calcium binding proteins, neuropetides have been used as markers, such as: neuropeptide Y (NPY), somatostatin (SOM), vasointestinal peptide (VIP) and cholecystokin (CCK) (Markram et al. 2004). Recently, the ionotropic serotonin receptor (5HT3aR) has also been used to classify interneurons (Rudy et al. 2011). In fact, populations expressing PV, SOM and 5HT3aR have different embryonic origins and account for nearly all cortical inhibitory cells, despite each class including various morphological and electrophysiological types of interneurons that are like to play different function within the circuits (Rudy et al. 2011). An extensive description of each marker expression is beyond the aim of this section, but a brief summary of the three populations expressing CBPs is here reported:

Parvalbumin-positive neurons: they comprise about 40% of the total inhibitory cell population and they are located in all layers of the neocortex except for layer 1 (Rudy et al. 2011). Parvalbumin is never co-expressed with somatostatin or calretinin, while in a small fraction of cells parvalbumin is co-expressed with calbindin (Kawaguchi and Kubota 1993). Morphologically, parvalbumin positive cells include both basket cells and chandelier cells (Markram et al. 2004).

Calbindin-positive neurons: they are located in layers 2 – 6, although they are more abundant in layer 2/3 (Markram et al. 2004). They include very different morphological classes: bi-tufted and Martinotti cells, but also minor fractions of basket cells and chandelier cells (Markram et al. 2004). They very often co-express somatostatin as well as parvalbumin and NPY (Gentet 2012).

Calretinin-positive neurons: they have been found in all layer of the neocortex, but the vast majority are located on the supragranular layers (Markram et al. 2004). Morphologically, they include: bipolar, bitufted and Martinotti cells. A large portion of these cells also express VIP, while only a small fraction co- expresses CCK (Markram et al. 2004).

1.3.2.1.2.3 Electrophysiological properties

In response to somatic depolarization, neocortical interneurons exhibit a great variety of firing patterns, which reflect: differences in passive membrane properties, densities and locations of voltage-dependent channels. Their characterization started with extracellular recordings in primate' neocortex (Mountcastle et al. 1969) and then continued and evolved with intracellular recordings in *in vitro* brain slices (Connors et al. 1982, McCormick et al. 1985). Initially, interneurons were classified as fast-spiking cells (McCormick et al. 1985), but later many different firing patterns have been discovered in inhibitory cells (Kawaguchi and Kubota 1993, Kawaguchi 1995, Kawaguchi and Kubota 1997). Two broad categories: fast-spiking interneurons (FS) and low threshold spiking interneurons (LTS) have been

used in the past and continue to be used today, even if a more refined classification has been set in place by the Petilla group in 2008 (Ascoli et al. 2008). According to the Petilla Group, neurons can be classified on the type of onset and steady – state firing pattern in response to a square pulse of depolarizing current (Ascoli et al. 2008). By looking at the onset type of firing, three classes have been indentified: continuous (where the initial response does not differ from the steady state firing behaviour); burst (where the initial instant firing frequency is higher compared with the steady state frequency); and delayed (where there is a delay between the time of initiation of the current injection and the firing onset that is greater than the expected based on the membrane (τ_m) (Ascoli et al. 2008). The steady state response can instead be classified in five classes: non-accommodating (continuous firing without frequency adaptation); accommodating (firing frequency adaptation); stuttering (high frequency firing interposed to periods of silence); irregular spiking (random single action potential firing spaced by variable periods of silence); and bursting (firing in clusters of 3-5 action potentials followed and preceded by long hyperpolarisation) (Ascoli et al. 2008). Although this type of classification is certainly useful, it is important to note that the firing pattern of the cell is also dependent on: the amount of current injected, and the temperature at which the recording are performed. Other electrophysiological factors can also be taken into consideration when classifying neuronal cells, such as passive membrane properties (resting membrane potential, membrane resistance, capacitance and time constant), action potential properties (spiking threshold, spike amplitude, half-width and afterhyperpolarization phase) and postsynaptic responses on the target cell (however those depend also on the postsynaptic cell identity) (Ascoli et al. 2008, Markram et al. 2004). In this thesis, interneurons will be broadly classified into two groups:

- Fast-spiking interneurons (FS): they are characterized by very high action potential firing rates (up to 400 Hz) with little or no frequency adaptation, fast and narrow action potentials, and brief, fast after hyperpolarisation (Gibson et al. 1999, Beierlein et al. 2003, Avermann et al. 2012). Based on the morphological traits,

those cells can be classified as basket cells and, less frequently, chandelier cells (Markram et al. 2004, Gentet 2012). They also express the calcium binding protein parvalbumin (Markram et al. 2004). They are found in all cortical layers except for layer 1, although their distribution within the layers is not uniform; “hot zones” at higher density have been found in layer 2 and 5A (Meyer et al. 2011).

- Non fast-spiking interneurons (NFS): this class includes all the inhibitory cells that did not meet the FS criteria. These cells have broader spikes, higher membrane resistance and lower firing threshold; do not reach high firing frequencies and show high frequency adaptation and amplitude accommodation. These cells are most commonly referred to as low threshold spiking (LTS) neurons.

1.3.2.2 Cell connectivity

A neural circuit can be defined as a set of cells functionally connected. A neocortical connection between two neurons generally consists of multiple synaptic contacts (Cheetham et al. 2007). The probability of two cells being connected is variable, and depends on the cell types considered and the developmental state of the brain. Pyramidal cells in layer 2/3 are sparsely interconnected (Feldmeyer 2012). Their probability of connection decreases with the increase in somatic distance between the two cells. In *in vitro* brain slices, the probability of excitatory connection between pyramidal cells of mature layer 2/3 primary somatosensory cortex is about 4% (Cheetham et al. 2007). Whether this low connectivity reflects a high degree of specificity, it is still matter of debate (Thomson and Lamy 2007, Kalisman et al. 2005, Fino et al. 2012). The probability of excitatory connection onto interneurons, estimated by paired cells recordings is, in contrast, much higher, and ranges between 20 - 70% (Thomson and Lamy 2007). Similarly, the probability of local inhibitory connections onto layer 2/3 pyramidal cells ranges between 20 and 60% with paired cell recordings, but recently measures of glutamate uncaging combined with two photon microscopy have revealed a connectivity of about 70% for both somatostatin-positive cells (predominantly Martinotti cells) (Fino and Yuste 2011) and parvalbumin-positive cells (FS interneurons) (Packer and Yuste 2011) in a 200 µm radius. Connectivity

between interneurons is also a function of the interneuron subtype. Moreover, inhibitory cells are also often connected not only by chemical, but also by electrical synapses (Gibson et al. 1999, Galarreta and Hestrin 1999). Parvalbumin-positive cells are highly interconnected by both chemical and electrical synapses in young and adult animals (Galarreta and Hestrin 2002). They also make and receive synaptic contacts to and from somatostatin - positive cells, with a probability of connection of about 50%, both ways (Ma et al. 2012). However, chemical connections between somatostatin-positive cells are extremely rare, although they are often interconnected by electrical synapses (Gibson et al. 1999, Hestrin and Galarreta 2005, Ma et al. 2012). Differences in specificity of contacts and in synaptic behaviour are likely to reflect different functional networks, although their specific roles in information processing have yet to be identified (Fino et al. 2012).

1.4 Barrel cortex plasticity

As I discussed in Section 1.3, the barrel cortex is an optimal system to study cortical map reorganization. However, it is extremely important to discriminate between reorganization induced by deafferentation (lesions) and sensory deprivation by whisker trimming. In the first case, the loss of input is more complete and the circuit's reorganization is likely to be mediated by different mechanisms (Barnes and Finnerty 2010). For example, forepaw denervation in adult animals has been shown to relocate the border of the corresponding cortical representation, and this process is associated to dendritic remodelling of pyramidal cells (Hickmott and Steen 2005, Hickmott and Ethell 2006). By contrast, non traumatic deprivation leaves dendritic arborisations of excitatory cells unaffected (Trachtenberg et al. 2002, Cheetham et al. 2008). Sensory deprivation and deafferentation also differ in the effect that they produce at different stages of the animal‘s life. For example, follicle ablation generates cortical map expansion of the representation of remaining whiskers (Kossut and Hand 1984) both in young and adult animals (Kossut et al. 1988). Moreover, limb deafferentation in adulthood has also been associated with plasticity of subcortical structures, like the thalamus (Jones and Pons 1998). By contrast, whisker trimming also alters the cortical map representation, but plasticity of thalamocortical afferents in layer 4 is

prominent in very young animals, but it then progressively decreases (Fox et al. 2002). In adulthood, plasticity is retained predominantly in cortical layer 2/3 and, to a lesser extent, in layer 5 (Fox 1992). There is, however, recent evidence showing structural alteration of thalamo-cortical axons in response to whisker trimming in adult animals (Oberlaender et al. 2012).

When studying experience-dependent plasticity, it is extremely important to consider the developmental stage of the animal. The mechanisms of adult plasticity are very likely to be different from the ones involved in the critical period of development. In fact, whereas cortical circuits are set in place and refined during development, adult plasticity requires modifying already established circuits (Albieri and Finnerty 2012). In the next section, I will discuss the development of the barrel cortex and the evidence for developmental plasticity, focusing specifically on inhibitory circuits.

1.4.1 Developmental plasticity

The formation and development of the maps is a really complex topic, since it involves a combination of both genetic factors and sensory experience (Feldman and Brecht 2005). The development and plasticity of primary somatosensory maps are layer specific (Stern et al. 2001). The first postnatal week is extremely important for the correct development of layer 4 maps. Follicles ablation at day 1 leads to the anatomical disruption of the barrels (Van der Loos and Woolsey 1973), consistent with thalamo-cortical connection plasticity. Lesion induced disruption of layer 4 barrels is, however, possible only before P4 (Woolsey and Wann 1976). Non-traumatic sensory deprivation does not affect barrel formation (Simons and Land 1987). In fact, chronic whisker trimming of the C row started at birth induces cortical changes in receptive fields of layer 4 cells, but it is not accompanied by cytoarchitectonical alterations (Simons and Land 1987). Plasticity in layer 4 is believed to decrease after P4, and by P7 the layer is not plastic anymore, as shown by single vibrissae experiments (Fox 1992). The first postnatal week has then been defined as the critical period of development for layer 4. However, recent evidence reopened the question

of whether synaptic plasticity could occur at thalamocortical connections after the end of the critical period (Oberlaender et al. 2012, Yu et al. 2012). Resection of the unilateral infraorbital nerve in 4 weeks old rats was reported to induce strengthening of the thalamocortical connections in spared cortex, as shown using manganese-enhanced fMRI and slice electrophysiology (Yu et al. 2012). A second study also showed that whisker trimming in adulthood strongly reduces the length of thalamocortical axons projecting into deprived cortex, leaving synaptic density unchanged (Oberlaender et al. 2012). Surprisingly, the magnitude of sensory evoked responses was not altered in deprived cortex whereas synchrony was increased in deprived cortex (Oberlaender et al. 2012). This suggests that other elements of layer 4 circuitry could possibly be altered by deprivation in adult animals (Oberlaender et al. 2012).

Whisker trimming starting at P7 can still affect the inhibitory circuits of layer 4 (Jiao et al. 2006). Whisker deprivation from P7 until P30, in fact, decreases the expression of parvalbumin in the inhibitory cells of deprived cortex (Jiao et al. 2006). Parvalbumin-positive cells also show a decrease in number of boutons and reduced mIPSC amplitude and frequency compared to control and spared cortex (Jiao et al. 2006). These effects cannot be elicited when trimming starts at P15 (Jiao et al. 2006).

The intracortical circuits of layer 2/3 develop following the barrels (Stern et al. 2001). This possibly reflects the order of cells migration in the cortical layers (Fox 2008). Maturation of layer 2/3 intracortical circuits occurs soon after P12 and is completed by P21 (Stern et al. 2001). The process coincides with both: the increase in number of (putative) excitatory and inhibitory synaptic contacts (Micheva and Beaulieu 1997, Micheva and Beaulieu 1996) and the beginning of active explorative whisking (~P11-13) (Welker 1964). While the increase in number of (putative) excitatory synapses increases progressively between P10 and P30, the increase in number of inhibitory contacts seem to slow down around P20, but then increase again until reaching maturity around P30 (Micheva and Beaulieu 1996).

1.4.2 Experience-dependent plasticity of receptive fields

Sensory deprivation by whisker trimming results in plasticity of receptive fields that leads to increased response to the spared whiskers and decrease response to the deprived ones (Fox 1992). The extent and type of the changes induced by experience deprivation in cortical activity are influenced by the type of whisker trimming protocols adopted. Different trimming protocols reveal dissimilar components of plasticity.

Receptive fields plasticity involves the potentiation of responses associated with spared input. When only one vibrissa is left, the potentiation takes 18 - 20 days to occur (Fox 1992). Conversely, when the whiskers are cut in a chessboard pattern the potentiating effect is much quicker, with changes recorded in just a week (Wallace and Fox 1999b). This suggests that a sort of co-operation exists between spared inputs, although the mechanism is still incompletely understood. Intracortical connections show plasticity after whisker trimming starting during development (P11) (Finnerty et al. 1999). Connections from spared to deprived cortex are strengthened and the synaptic dynamics show an increased depression compared with deprived to deprived connections (Finnerty et al. 1999). When sensory deprivation starts at P19 and lasts for weeks the excitatory connections between layer 2/3 pyramidal neurons in spared cortex are potentiated, in a process that does not involves increase of synaptic contacts (Cheetham et al. 2007). The functional increase in strength is associated with the structural enlargement of both the presynaptic axonal terminals and the postsynaptic spines (Cheetham et al. 2012). A possible mechanism of synaptic strengthening is long term potentiation (LTP). LTP can be induced in layer 2/3 of adult animal (Castro-Alamancos et al. 1995, Glazewski et al. 1998a, Hardingham et al. 2003), it is both NMDA (Castro-Alamancos et al. 1995) and CAMKII dependent (Hardingham et al. 2003) but, unlike hippocampal LTP, it also consists of a prominent presynaptic component (Hardingham and Fox 2006). The GluR1 subunit of AMPA receptor is also important for postsynaptic plasticity in neocortex (Hardingham and Fox 2006). Although there is no definitive proof that deprivation induced strengthening occurs through LTP, both processes involve CAMKII (Hardingham et al. 2003). A recent

study has demonstrated that excitatory synapses on layer 2/3 pyramidal cells of P21 - P51 mice are potentiated by pairing whisker stimulation with postsynaptic action potential firing (when the action potential is fired 10 ms after the whisker-evoked PSP) (Gambino and Holtmaat 2012). The form of synaptic plasticity in which the direction of the change in strength of a connection is established by the spiking order of the pre and postsynaptic neurons within a specific time window is defined as spike-timing dependent plasticity (STDP) (Markram et al. 1997, Markram et al. 2011, Feldman 2012). In a control animal only the deflection of the principal whisker generates spike timing dependent plasticity in neurons that are in the centre of the principal whisker receptive field (Gambino and Holtmaat 2012). However, when all but two whiskers are trimmed for 2 - 3 days, stimulation of the surrounding whisker followed by action potential also generates spike-timing dependent potentiation. Another extremely important effect of sensory deprivation has been discovered recently by performing multi-photon long term imaging of layer 2/3 pyramidal cells genetically expressing calcium indicator *in vivo* (Margolis et al. 2012). In response to whisker stimulation, cells respond with different levels of activity, which are stable across trials. When all but one whisker are trimmed for just 2 - 3 days: the responses to the deprived whiskers are generally decreased, while the responses to the spared whisker change, with the cells that were less active becoming more active and viceversa, both in spared and deprived cortex (Margolis et al. 2012). These results suggest that cortical reorganization induced by whisker trimming involves a reorganization of excitatory circuits that results in change in population outputs. Interestingly, recordings of synaptically connected pairs of neurons in deprived cortex in brain slices after 2 - 3 days of deprivation started at P30 reveals a temporary increase in excitatory connectivity, from 4% to 12% (Barnes 2010). The increase in probability of excitatory connection could reflect the process of rewiring that underlies the change in population output.

Receptive field plasticity also involves depression of responses associated with the missing input. Trimming all whiskers generates depression of the responses mainly in cortical layers 2/3 after whisker re-growth (Glazewski et al. 1998b). In contrast, no changes

are detected in the thalamus (Glazewski et al. 1998b). However, the unilateral removal of all the whiskers after the end of the critical period induces only negligible changes in layer 2/3 cell responses (Stern et al. 2001). In contrast, when two whiskers are left, plastic changes in layer 2/3 are recorded after only 24 hours (Diamond et al. 1994), suggesting that competitive cortical activity is required for plasticity. In the chessboard trimming protocol, in which every deprived column is surrounded by four spared ones, the depression of responses in the cortex is greater than the one recorded with the univibrissa protocol (Glazewski et al. 1998b, Wallace and Fox 1999a). This suggests that, in addition to the direct effect of stimuli deprivation, a secondary component of depression is generated by the activity of surrounding whiskers. Blocking cortical activity in a chessboard trimmed animal by applying muscimol to the cortical surface results in reduced depression of responses in deprived cortex (Wallace et al. 2001). The depression of deprived whisker is associated with an LTD like depression of intracortical connection between layer 4 and layer 2/3 when whisker plucking starts at P12 and last for 10 – 20 days (Allen et al. 2003). Deprivation also alters the spiking order between layer 4 and layer 2/3 cells, with cells in layer 2/3 spiking before cells in layer 4 (Celikel et al. 2004). This change in spiking order is consistent with a mechanism of LTD induced by spike timing dependent depression (Allen et al. 2003). The weakening of layer 4 to layer 2/3 synapses is likely to be the result of a presynaptic decrease of probability of release, as it is associated with an increase of pair pulse ratio (Bender et al. 2006). However, neither the amplitude nor the frequency of miniature EPSC in layer 2/3 deprived pyramidal cells was altered by deprivation (Bender et al. 2006).

Experience-dependent plasticity in the period that follows the reaching of sexual maturity occurs without the depression of deprived inputs that has been recorded in adolescent animals (Glazewski and Fox 1996). The potentiation of spared inputs seems to be the only mechanism that persists in maturity (Fox 2002).

1.4.3 The role of inhibition in shaping cortical receptive fields

In the previous section I described the changes in receptive fields induced by sensory deprivation and the possible cellular mechanisms by which excitatory circuits are altered. However, inhibitory circuits are also involved in shaping receptive fields (Bruno and Simons 2002, Brumberg et al. 1996). There is large evidence that blocking locally $GABA_A$ receptors in vivo not only increases the magnitude of the evoked responses, but also enlarges the receptive field dimensions of the corresponding sensory input (Wirth and Luscher 2004, Foeller et al. 2005). The role of inhibition however is not confined to the boundaries of the principal column.

Extracellular recordings from rats exploring a mesh screen shows an average 20% increase in firing activity of the principal whisker immediately after trimming of the adjacent whiskers (Kelly et al. 1999). These results have been interpreted as the proof of inhibitory role of adjacent whiskers (Kelly et al. 1999). In the intact whisker system, stimulation of adjacent whiskers has an inhibitory effect on the principal whiskers. After trimming adjacent whiskers and, therefore, removing the source of inhibition, the activity of the principal whisker is increased. Furthermore, when gabazine (GABAa antagonist) is injected in layer 2/3 of a control animal, the surrounding whiskers responses are increased more compared with the principal whisker, indicating that dishinibition is stronger for the surrounding whiskers and it facilitates receptive field expansion (Foeller et al. 2005). However, gabazine administration to animals that undergo univibrissa experience starting from P13 and lasting for 14 - 21 days induces a different effect: the bigger disinhibition is now associated with the deprived principal whisker (Foeller et al. 2005). This result is compatible both with: a possible increase of inhibitory conductances in the deprived area that facilitates the sharp tuning of the spared vibrissa or alternatively, inhibition could be unchanged and the decrease in dishinibition of surrounding whiskers would just reflect changes in the excitatory circuits (Foeller et al. 2005).

1.4.4 Inhibitory circuits gate the critical period of development

Inhibitory circuits are fundamental during the critical period of plasticity (Hensch and Fagiolini 2005). Their role has been studied most extensively in visual cortex. The deprivation paradigm that best corresponds to whisker trimming in visual cortex is monocular deprivation. This paradigm consists of occluding one eye for a specific time period. Monocular deprivation, when it is performed during development, results in an ocular dominance shift, with preferential tuning of binocular cortex cells (or columns, in some species) towards the open eye (Hensch 2005). Although the cellular mechanisms are likely to be different, monocular deprivation also induces an ocular shift in adulthood (Hofer et al. 2006). The role of inhibitory circuits in controlling the critical period was shown initially in GAD65 knock-out mice (Hensch et al. 1998, Hensch 2005). GAD65 is the enzyme that synthesizes GABA in the axonal terminals. The absence of GAD65 prevents ocular dominance plasticity (Hensch et al. 1998). The onset of plasticity can also be delayed by rearing animals in complete darkness from birth (Morales et al. 2002). Conversely, the critical period of plasticity can be anticipated by the administration of benzodiazepines, which increase GABA transmission (Fagiolini and Hensch 2000). The administration of BDNF, which induces interneuron maturation, also brings forward the beginning of the critical period (Hensch 2005). Parvalbumin-positive fast-spiking interneurons play a major role in gating the critical period of development (Hensch 2005). The potentiation of responses in the open-eye cortex could be explained by both; LTP of the thalamocortical excitatory synapses as well as by LTD of the inhibitory drive on the excitatory circuit (Yoshimura et al. 2003). Similarly, the rapid decrease in responsiveness of the deprived eye could potentially be obtained by direct depression of the excitatory synapses or by the potentiation of the inhibitory drive. Two days of monocular deprivation during the critical period do not affect synaptic transmission between star pyramid cells (Maffei et al. 2006). However, the feedback inhibitory circuits comprising star pyramid cells and fast-spiking interneurons are potentiated (Maffei et al. 2006). This is reflected in a tenfold reduction of layer 4 star-pyramids spontaneous firing (Maffei et al. 2006).

The great importance of the role played by interneurons during critical period plasticity has been shown by Southwell et al. (Southwell et al. 2010). To assess the role of interneurons in gating the critical period of development, embryonic inhibitory neurons precursors were transplanted into the visual cortex of animals at P0 - 2 and P9 - 11 (Southwell et al. 2010). In response to 3 - 4 days of monocular deprivation, those animals showed both; a normal critical period of plasticity which reaches a peak around P33 - 35, but also a second period of high plasticity around P45, when the transplanted cells reach the age of their endogenous normal critical period of plasticity (Southwell et al. 2010). The transplanted cells receive and form new connections with excitatory host cells, which are more abundant but less strong than the number and strength of normal connection (Southwell et al. 2010). This study strongly suggests that interneurons are sufficient to gate cortical period plasticity. However, in order to prove definitively this hypothesis, it would be necessary to show that interneuron transplantation can induce a second critical period in an animal that has already completed its own critical period of development (> P45 - P50).

1.5 Is there evidence for disinhibition in cortical map plasticity?

1.5.1 Functional plasticity

In the previous two sections I presented the evidence for a role of inhibition in shaping receptive fields (see Section 1.4.3) and gating the critical period of plasticity (see Section 1.4.4). However, there is still little evidence that inhibition is actively involved in the process of cortical map plasticity in adulthood.

It has been proposed that the immediate changes in receptor fields associated with the missing input are attributable to a decrease in intracortical inhibition in the surrounding area (Calford and Tweedale 1988, Garraghty et al. 1991, Jones 1993, Lebedev et al. 2000). This hypothesis has been supported by immunostaining studies of GABA and GAD

expression (Jones 1993). In adult monkeys, monocular deprivation induced by eye enucleation, TTX injection or eye lid suture induces a reversible decrease in the number of cells and terminals positive for GABA and GAD in layer 4, and to a lesser extent in layer 2/3 (Hendry and Jones 1986, Hendry and Jones 1988).

In barrel cortex, whisker trimming of the C row in adult rats (> than 2 months old) for 5 – 10 weeks also alters the expression of GAD (Akhtar and Land 1991). GAD immunoreactivity is decreased in layer 4 and also in layer 2/3 of deprived cortex. However, the pattern of GAD expression is altered also in the non deprived barrels, with an increase in staining of layer 2/3 in the spared area closest to the deprived barrel (Akhtar and Land 1991).

Changes in feed forward inhibition from layer 4 have been detected in rats when whisker trimming starts during the critical period (P12) and lasts for 6 – 12 days (House et al. 2011). Deprivation reduces the strength of feedforward inhibition on fast-spiking interneurons of layer 2/3 deprived cortex (House et al. 2011). The reduction in feedforward inhibition is even bigger than the decrease in feedforward excitation on pyramidal neurons and it is balanced by a corresponding increase in amplitude of uIPSP within the local circuits of layer 2/3 in deprived cortex (House et al. 2011). The increase in strength of inhibitory synapses onto pyramidal cells occurs together with increase in connectivity between FS interneurons and pyramidal cells (House et al. 2011). The authors concluded that the decrease in feed forward inhibition from layer 4 is offset by the increase in strength of local inhibitory connections in order to maintain the balance between excitation and inhibition (House et al. 2011). To my knowledge, no study has yet reported electrophysiological evidence of functional changes in the inhibitory circuitries as a result of sensory deprivation in barrel cortex after the end of the critical period. Nonetheless, a few studies have recently reported structural evidence indicating that inhibitory circuits are plastic in adulthood during cortical map reorganization (Chen et al. 2011, Marik et al. 2010). Structural plasticity will be extensively discussed in Section 1.5.3.

Functional alteration of the balance between excitation and inhibition has been reported during receptive field plasticity *in vivo* in primary auditory cortex (Froemke et al. 2007). Pairing stimulation of the nucleus basalis with a frequency specific tone alters the tuning of individual neurons, shifting their preferred frequency towards the played tone (Kilgard and Merzenich 1998). The receptive field changes are associated with a rapid decrease of inhibition and increase of excitation (Froemke et al. 2007). This phase of transitory disinhibition could enable reorganization of excitatory circuitry. This initial phase is then followed by a gradual increase in inhibition over the next hours, which restores the excitatory - inhibitory balance (Froemke et al. 2007). Therefore, local, transient disinhibition induced by neuromodulation could be a possible mechanism by which the excitatory circuits are allowed to reorganize functionally.

1.5.2 Mechanisms of plasticity within the inhibitory circuits

The role of inhibitory circuits in cortical map plasticity has received very little attention compared with excitatory circuitry. This can in part be attributed to the higher complexity and heterogeneity of inhibitory subclasses, but it is also related to the belief that inhibitory circuits are not plastic. However, recent studies have shown how both excitatory connections onto interneurons and inhibitory connections onto excitatory cells undergo different forms of plasticity (Kullmann et al. 2012).

In general, there are only a few categories of cellular plasticity mechanisms that can alter the output of neural circuits. These include: changes in intrinsic excitability, changes in synaptic strength of connection between neurons and rewiring (Albieri and Finnerty 2012, Barnes and Finnerty 2010). Although the number of plasticity mechanisms is limited, they can coexist and interact with each other at each connection. The number of connections in neocortex and the fact that inhibitory and excitatory cells are strongly interconnected, makes it is extremely hard to understand the mechanisms underpinning cortical map reorganization. In the next sections I will describe the plasticity mechanisms that alter

inhibitory circuits, with particular attention to those that are involved in cortical map plasticity.

1.5.2.1 Intrinsic excitability

The spatio-temporal combination of excitatory and inhibitory drive into a cell determines the ability of a cell to fire action potentials. However, the probability of evoking an action potential is also determined by the intrinsic properties of the cell itself. By altering those properties, experience-dependent plasticity and learning and memory can modify neuronal circuits and, at least theoretically, store new memories (Zhang and Linden 2003).

Even though a few studies reported changes in intrinsic excitability of excitatory cells in response to sensory deprivation, only one study, to my knowledge, investigated the effect of whisker trimming on the intrinsic properties of interneurons (Sun 2009). Removing the D row of whiskers from P7 until P30 alters the firing properties of layer 4 FS interneurons in deprived barrel cortex. As result of deprivation, the firing frequency decreases, the firing threshold is more positive and the input-output function of FS interneurons in deprived cortex is right-shifted. Although the passive membrane properties of FS interneurons are also significantly affected, with a decrease input resistance (R_m) and increase in membrane time constant (τ_m), the shift of the input output function is mainly associated with an increase in voltage-dependent potassium channel current I_A (Sun 2009). This change in intrinsic excitability is selective for fast-spiking interneurons cells, as none of the above changes has been reported in non fast-spiking (NFS) cells (Sun 2009). At present however it is still not clear whether the intrinsic excitability of interneurons can be affected by experience-dependent plasticity in adulthood.

1.5.2.2 Plasticity of excitatory synapses onto interneurons

Although long term potentiation (LTP), the activity-dependent strengthening of synaptic transmission, was initially discovered at excitatory synapses on pyramidal neurons, there is growing evidence that it also occurs at excitatory synapses onto interneurons (Kullmann

et al. 2012). LTP of excitatory synapses on interneurons have been largely investigated in hippocampal brain slices. The difficulties in identifying LTP of connections onto interneurons are also related to the high diversity of interneuron subtypes that not necessarily share the same plasticity mechanisms. LTP can be induced in some interneuron subtypes in the stratum oriens of the CA1 by theta burst stimulation associated with postsynaptic depolarization (Kullmann and Lamsa 2011, Lamsa et al. 2010). This form of LTP requires metabotropic glurtamate subtype receptor 1a and is NMDA receptor-independent. However, in some interneuron subtypes of the stratum oriens, LTP can also be induced by combining theta burst stimulation with postsynaptic hyperpolarization (Lamsa et al. 2005, Oren et al. 2009). This form of LTP, which has been described as “anti Hebbian”, requires calcium permeable AMPA receptors and it is also NMDA-receptor independent (Lamsa et al. 2007). Excitatory connections from the Shaffer collaterals onto interneurons of the stratum radiatum are instead NMDA-receptor dependent, and they share many properties of LTP in excitatory cells (Kullmann and Lamsa 2011). Although, a notable difference is that interneurons do not express CAMKIIalpha (Kullmann and Lamsa 2011). Evidence of long term depression (LTD) of excitatory synapses has also been found on interneurons of the CA3 area (Kullmann and Lamsa 2011).

Although LTP and LTD of excitatory connections on interneurons has been mainly investigated in hippocampus, there are several studies that show how those forms of synaptic plasticity are also present in other brain areas, such as amygdala, striatum, brainsteam and neocortex (Kullmann and Lamsa 2011, Kullmann et al. 2012). In mouse visual cortex, LTP of excitatory connections on layer 2/3 FS interneurons can be induced by pairing layer 4 theta burst extracellular stimulation with postsynaptic depolarization (Sarihi et al. 2008). The potentiation is NMDA receptor independent, is affected by the amount of postsynaptic calcium and is dependent on metabotropic glutamate receptors mGluR5 (Sarihi et al. 2008). LTP has also been induced in somatostatin-positive interneurons of somatosensory cortex (Chen et al. 2009). This LTP mechanism is also NMDA-receptor independent, but, unlike LTP of excitatory inputs on FS interneurons, it

does not depend on postsynaptic calcium and it is likely to be expressed presynaptically (Chen et al. 2009).

1.5.2.3 Spike timing dependent plasticity

Changes in strength of excitatory connections on layer 2/3 FS interneurons of somatosensory cortex can also be induced by spike timing dependent plasticity protocols. In excitatory connections between pyramidal cells, the type of change in synaptic strength (LTP or LTD) has been found dependent on the spike order and timing of pre and postsynaptic firing within a short time-window (Markram et al. 1997). This mechanism of synaptic plasticity has been therefore described as spike timing dependent plasticity (STDP). In excitatory cells, when the presynaptic cell spikes about 10 - 20 ms before the postsynaptic cell, the connection undergoes LTP, while LTD is induced when the order is reversed and the postsynaptic cell fires between 20 and 200 ms before the presynaptic neuron. STDP has been shown to occur at many different synapses, although the rules for induction vary according to different brain areas and cell types (Dan and Poo 2004, Markram et al. 2011, Feldman 2012). Plasticity of excitatory connections onto interneurons also can be induced with a STDP protocol (Kullmann et al. 2012). In neocortex, one study has shown that in young rat (P13 - P16) primary somatosensory cortex, excitatory conections on layer 2/3 regular spiking interneurons obey to the classical rules of STDP, with potentiation induced by a “pre before post” paradigm and, vice versa, depression by “post before pre” stimulation (Lu et al. 2007). However, these rules do not apply to FS interneurons in the same cortical layer, where both “pre before post” and post before pre” paradigms induce LTD only (Lu et al. 2007).

1.5.2.4 Plasticity of inhibitory synapses onto pyramidal neurons

Bidirectional changes in transmission strength have been reported at GABAergic synapses in different areas of the brain (Castillo et al. 2011). The mechanisms of plasticity at inhibitory synapses are multiple and they are mediated both pre and postsynaptically (Castillo et al. 2011).

One of the best characterised presynaptic forms of plasticity at GABAergic synapses in the endocannabinoid - mediated LTD (Castillo et al. 2011, Kullmann et al. 2012). The release of endocannabinoids from the postsynaptic cell is induced by presynaptic activity of the interneuron combined with activation of metabotropic glutamate receptors by glutamate released by excitatory synapses onto the postsynaptic neuron. Endocannabinoid receptor activation in the presynaptic bouton reduces the synaptic efficacy of the GABAergic connection. This type of LTD has been reported initially in amygdala and hippocampus, but it occurs in also in the developing visual cortex, where it has been suggested to be part of the inhibitory synpase maturation process (Jiang et al. 2010). Potentiation of inhibitory synapses has also been reported in developing visual cortex (Inagaki et al. 2008). The candidate retrograde messenger involved in LTP of GABAergic synapse induced by presynaptic high frequency stimulation is the neurotropic factor, BDNF, which is released postsynaptically and then activates TrkB receptor signalling in the presynaptic neuron (Inagaki et al. 2008). GABAergic activity can also be altered postsynaptically by affecting $GABA_A$ receptors function, insertion/removal and trafficking (Castillo et al. 2011). Postsynpatic inhibitory LTP can be induced in layer 4 of developing visual cortex (P21 – P24) by pairing trains of action potentials at 50 Hz in the FS interneuron with the postsynaptic sub-threshold depolarization of the star pyramidal cell (Maffei et al. 2006). The phenomenon is associated with an increased in number of opened channels (Maffei et al. 2006). Induction of plasticity is prevented by monocular deprivation (Maffei et al. 2006). The signalling cascade activated by this process is however unknown and the mechanisms of detection of pre- and postsynaptic activity still need to be elucidated.

1.5.2.5 Experience-dependent synaptic plasticity: spontaneous and miniature transmission

The effect of experience-dependent plasticity on connection strength can be investigated by recording miniature excitatory postsynaptic potentials/currents (mEPSP/mEPSC). Miniature activity conveys a measure of the overall excitatory drive onto interneurons.

Changes in mEPSP/C amplitude or frequency are markers for circuits' plasticity, although it can be difficult to infer much about the mechanisms by which the changes occur, because changes cannot be attributed to specific inputs (Cheetham et al. 2007). Despite these limitations, spontaneous and miniature postsynaptic events have proven useful in the initial assessment of changes induced by experience-dependent plasticity.

In visual cortex, two days of monocular deprivation started during the pre-critical period decreases the spontaneous inhibitory postsynaptic current (sIPSC) amplitude in layer 4 pyramidal cells (Maffei et al. 2004). However, the same protocol of monocular deprivation performed during the critical period induces strengthening of the layer 4 feedback inhibitory circuits involving FS interneurons and pyramidal cells (Maffei et al. 2006). In layer 2/3, monocular deprivation induced during the critical period by TTX injection increases the total spontaneous excitatory drive on pyramidal cells and decreases the spontaneous inhibitory postsynaptic current (sIPSC) charge (Maffei and Turrigiano 2008). However, lid suture at the same time point decreases the excitatory drive but leaves the inhibitory drive unaltered (Maffei and Turrigiano 2008). Changes in miniature transmission in visual cortex have also been found after permanent focal retinal lesion in adult mice (Keck et al. 2008, Keck et al. 2011). This paradigm generates a temporary loss of responsiveness in the corresponding portion of monocular cortex, which reorganizes structurally and functionally (Keck et al. 2008, Keck et al. 2011). mIPSC frequency is decreased in layer 5 pyramidal cells in animals whose retina has been completely lesioned, which supports the structural decrease in bouton density in the axonal arbors of layer 2/3 spiny interneurons (Keck et al. 2011).

In barrel cortex, a long period of sensory deprivation (whisker trimming of a single row of whiskers for 23 days) starting during the first postnatal week decreases the frequency of sEPSCs in FS interneurons of layer 4 whereas sEPSC amplitude is unaffected (Sun 2009). In contrast, a similar protocol of sensory deprivation starting at P19 increases miniature

IPSC amplitude in L2/3 pyramidal neurons of both spared and deprived cortex, while mIPSC frequency is not changed by deprivation (Edwards 2008).

1.5.2.6 Experience-dependent synaptic plasticity: evoked responses

A number of investigations have probed the relationship between altered sensory experience and the properties of evoked responses. In response to the firing of an action potential in the presynaptic neurons, a postsynaptic current is induced in the connected partner. The strength of the connection is determined at least by three factors: the number of synaptic contacts that form the connection; the quantal size (the size of the depolarization/hyperpolarisation induced by the release of a single vesicle of neurotransmitter); and the probability of neurotransmitter release at each synapse (Branco and Staras 2009). Moreover, the size of the postsynaptic current is dynamically regulated by the previous activity in the presynaptic neuron and the frequency of presynaptic action potential firing (Beierlein et al. 2003). The depressing (decrease in postsynaptic current amplitude) or facilitating (increase in postsynaptic current amplitude) behaviour of a connection in response to a train of action potential is believed to be related to changes in the probability of release, which is determined presynaptically (Atwood and Karunanithi 2002). Synaptic strength can also be altered by changes in quantal size. Quantal size could be affected presynaptically by changing the amount of neurotransmitter released by each vesicle or by its diffusion rate (Atwood and Karunanithi 2002). At the postsynaptic site, the quantal size can be increased or decreased by changing the number or functional properties of postsynaptic receptors (Atwood and Karunanithi 2002). Recording simultaneously two neurons that are synaptically connected allows to study in details the properties of a single-axon connection. It is important to remember that each neocortical connection generally consists of multiple synapses (Cheetham et al. 2007). This technique is however technically demanding, and in many studies evoked release has been induced by extracellular stimulation of multiple afferents. Extracellular stimulation, even minimal, has the big disadvantage of activating multiple inhibitory and excitatory afferents, including axons of cells in different layers, which could be driven to fire by antidromic propagation.

Polysynaptic activation can be common during extracellular stimulation and is usually attenuated pharmacologically or by altering extracellular ion concentrations. These considerations aside, evoked responses are a powerful way to investigate circuit plasticity in cortical map reorganization.

Experience-dependent plasticity has been shown to alter both evoked excitatory responses on interneurons and evoked inhibitory responses on pyramidal cells. In visual cortex, monocular deprivation during the pre-critical period (P15 - P17) strongly reduces the unitary amplitude of IPSC generated by FS interneurons onto star pyramidal neurons in layer 4 (Maffei et al. 2004). Conversely, when the animals are monocularly deprived during the critical period of plasticity (P18 - P21), both the excitatory connections between star-pyramid cells and FS interneurons and the inhibitory connections from FS interneurons onto star pyramid cells are potentiated in deprived cortex (Maffei et al. 2006).

In barrel cortex, changes in the feedforward inhibitory circuits have been reported when sensory experience is altered during the critical period of plasticity in layer 2/3 (House et al. 2011). When trimming starts at P12 and lasts for 6 to 12 days, the strength of layer 4 excitatory input onto layer 2/3 FS interneurons is significantly decreased (tested with extracellular stimulation), even more than the excitatory input onto principal cells (House et al. 2011, Allen et al. 2003). Moreover, the inhibitory connections from FS interneurons onto layer 2/3 pyramidal cells are strengthened (paired recordings) in the deprived area and the connectivity is also increased (House et al. 2011). However, no study has yet reported changes in evoked responses within inhibitory circuits in adulthood following sensory deprivation.

1.5.3 Structural plasticity

Although the evidence of functional changes in the inhibitory circuitry during cortical map plasticity in adulthood has not yet been provided, a few studies have recently imaged

structural plasticity of inhibitory circuits in response to sensory deprivation (Chen et al. 2011, Marik et al. 2010).

Passive whisker stimulation of a whisker row for 24 hours has been shown to induce a decreased metabolic response in the corresponding barrel (Welker 1992) and increase in GAD expression (Welker 1989). The same passive stimulation protocol also induced an increase in both excitatory and inhibitory synapses onto spines and a general increase in spine density and spines with two synapses (one excitatory and one inhibitory) in pyramidal cells of layer 4 (Knott et al. 2002). Both synapse and spine densities are back to control values 4 days after the stimulation, but the number of GABAergic synapses on spines is maintained as high as immediately after the stimulation (Knott et al. 2002).

The search for structural plasticity of cortical cells has been made possible by the development of two photon microscopy, which allows neurons to be imaged chronically *in vivo*. Dendrites of inhibitory cells, unlike excitatory pyramidal cells, undergo continuous protrusion and retraction of branches even in adulthood in layer 2/3 of visual and somatosensory cortex (Lee et al. 2006, Lee et al. 2008). Monocular deprivation induces a threefold increase in dendritic tips dynamics of later 2/3 GABAergic interneurons of binocular visual cortex (but not in monocular visual cortex) (Chen et al. 2011). The increase in dynamics occurs 4 days after deprivation, it persists at 7 days but it starts to fade off between 7 and 14 days (Chen et al. 2011). Layer 2/3 interneurons, between 0 and 4 days of monocular deprivation, undergo a phase of branch tip retraction that is followed in the next 3 - 4 days by a significant increase of elongations (Chen et al. 2011). The initial phase of retraction is also present after binocular deprivation, suggesting that this process could reflect the change in sensory input (Chen et al. 2011). However, there is no change in excitatory synapses density on interneurons after 0 - 4 days of deprivation. Therefore, synapse loss could be due only to the retraction of dendritic branches (Chen et al. 2011). In the same time period, interneuron axonal arbors are affected by boutons loss, which is reflected in the decrease of inhibitory synapses onto pyramidal cells of layer 5 (but not of

layer 2/3) (Chen et al. 2011). Interestingly, imaging of animals treated with fuoexetine shows increased dendritic tip dynamics under normal vision while under brief monocular deprivation the initial phase of retraction is substituted by a phase of elongation similar to the one observed 4 - 7 days after the start of deprivation in untreated animals (Chen et al. 2011). The structural effect of fluoxetine on inhibitory circuits could then, at least partially, explain its ability to restore plasticity in adult animals (Chen et al. 2011, Maya-Vetencourt et al. 2008).

Structural changes in inhibitory circuits have also been identified in visual cortex after focal retinal lesion, a paradigm that induces cortical map reorganization in the corresponding area of monocular visual cortex (Keck et al. 2008, Keck et al. 2011). This type of deprivation is not reversible and therefore it is more comparable to a peripheral lesion or to deafferentation than to innocuous sensory deprivation. Loss of visual input induced by focal retinal lesions affects spiny cortical interneurons (Keck et al. 2011). Interneuron spines have turnover rates similar to the ones reported for excitatory spines and receive mainly excitatory synapses (Keck et al. 2011). As a result of focal lesion, the number of inhibitory spines in layer 2/3 interneurons is significantly decreased in the 72 hours following the lesion (Keck et al. 2011). The decrease is stable over the next two months and it occurs not just in the deprived area, but also in the surrounding cortex (Keck et al. 2011). The loss of excitatory synapses on spiny interneurons precedes a significant decrease in interneuron boutons density and inhibitory synapses on layer 5 pyramidal neurons. These data suggest that a retinal lesion reduces the level of inhibition, possibly to balance the reduction in excitatory input. These effects are however restricted to a selective subset of interneurons, and it is still unclear whether similar changes occurs also in aspiny interneurons.

Further evidence that supports the existence of a window of disinhibition comes from chronic two-photon imaging of inhibitory synapses onto layer 2/3 pyramidal cells after monocular deprivation in adult mice (van Versendaal et al. 2012). Inhibitory synapses are

identified using GFP–tagged gephyrin, a scaffold protein specific to inhibitory postsynaptic densities. Monocular deprivation in adulthood reduces the number of inhibitory synapses onto spines located on distal apical dendrites of layer 2/3 pyramidal cells after just one day of deprivation and it persists for the following week (van Versendaal et al. 2012). Reopening of the eye is associated with a further decrease in the number of inhibitory synapses on spines (van Versendaal et al. 2012). The loss of inhibitory spines could facilitate the strengthening of excitatory intracortical or thalamic input, consistent with the evidence that monocular deprivation in adulthood occurs mainly by strengthening of non-deprived eye responses. However, it is not clear how recovery from monocular deprivation could involve further inhibitory synapses loss.

A recent imaging study by Marik *et al.* reveals how experience-dependent plasticity in adulthood also alters axonal dynamics in primary somatosensory cortex (Marik et al. 2010). The temporal evolution of cortical map expansion after whisker plucking of the D and E rows correlates with changes in axonal dynamics of both excitatory cells and inhibitory interneurons of layer 2/3 in spared and deprived cortex (Marik et al. 2010). After 14 days of plucking, the axons of excitatory cells in spared cortex show a significant growth towards the deprived area (Marik et al. 2010). However, inhibitory circuits also show deprivation induced changes, which occur in a shorter time scale. Two days of deprivation increases bouton turnover of interneurons in spared cortex, but their arborisations, although dynamic, do not grow towards the deprived area (Marik et al. 2010). The biggest axonal reorganization affects the interneurons located in the deprived area (Marik et al. 2010). However, two days of whisker plucking differentially affects axonal arborizations of interneurons in deprived cortex, which can then be divided in three classes: a subset of axons remain stable in length but decrease their bouton density; axons close to their soma retracts, and a final fraction of axons that extend toward the spared cortex increase their length (Marik et al. 2010). The increase in length of those axons is stable and persists after 30 days of deprivation (Marik et al. 2010). Therefore, sensory deprivation affects the axonal dynamics not only by increasing the turnover rate in

both excitatory and inhibitory cells but also by altering the structure of axonal arborisations. Interestingly, the inhibitory circuits show a higher level of structural plasticity that develops over a shorter time scale (changes can be seen even hours after whisker trimming) than excitatory neurons. Moreover reorganization of axonal arborisations is more pronunced in interneurons of deprived cortex. Hence, inhibitory circuits are still plastic in adulthood. The changes in axonal structure and bouton densities are likely to reflect functional alteration of the inhibitory circuitry, which still need to be elucidated.

1.6 Can plasticity be restored in adulthood?

Understanding the mechanisms underpinning cortical reorganization is extremely important as inducing and accelerating plasticity in adulthood could help the recovery from many neurological conditions.

Plasticity is reduced in adulthood compared with development. However, there is evidence that plastic changes can still occur after the end of the critical period (Glazewski and Fox 1996, Hofer et al. 2009). In visual cortex, for example, monocular deprivation induces ocular dominance plasticity in cortical responses mainly during the critical period (Levelt and Hubener 2012). However some small changes can be induced in adult mice after a longer period of monocular deprivation (Hofer et al. 2006).

Plasticity can be restored to higher level in adult visual cortex by the administration of the antidepressant fluoxetine, which can induce ocular dominance plasticity in adult rats and restore visual functions in adult amblyopic animals (Maya-Vetencourt et al. 2008). The administration of this antidepressant is associated with a decrease in extracellular GABA levels and an increased production of BDNF, whose cortical infusion per se can induce ocular dominance plasticity in adult rats (Maya-Vetencourt et al. 2008). The fluoxetine-induced plasticity is completely abolished by the treatment with diazepam suggesting a

strong link between plasticity and reduction of cortical inhibition, which might allow the reorganization of the circuitries (Maya-Vetencourt et al. 2008).

Ocular dominance plasticity can be restored in old animals also by the administration of choindroitinase-ABC, an enzyme that breaks down chondroitin sulphate proteoglycans (CPGs) (Pizzorusso et al. 2002). CPGs are part of the extracellular matrix and assemble in specialized structures called perineuronal nets. The disintegration of perineural nets, which become thicker and diffuse with the increasing of age, could facilitate the adult cortical experience-dependent plasticity allowing structural changing in substrates that were not accessible previously and axonal rewiring (Pizzorusso et al. 2002). The ability to undergo plastic changes in adulthood after the disruption of perineuronal nets represents also a further evidence of the importance of fast-spiking interneurons in the plasticity mechanisms, since perineuronal nets are particularly abundant around parvalbumin-positive cells (Pizzorusso et al. 2002). However, a recent study has shown that administration of chondroitinase in binocular visual cortex of cat monocularly deprived from the beginning of the critical period until ~ P105 did not induce a level of plasticity sufficient to reach a complete functional recovery (Vorobyov et al. 2013).

Recently a few molecules have also been shown to play a fundamental role in restoring adult experience-dependent plasticity. The Paired-immunoglobulin–like receptor B (PirB) is a major histocompatibility complex class I (MHCI) receptor, which is expressed in subsets of neurons throughout the brain (Syken et al. 2006). PirB is localised at synapses and mice carrying a mutated PirB that lacks of the transmembrane domain (and therefore is unable to transduce signals) have increased ocular dominance (OD) plasticity both during and after the critical period of plasticity (Syken et al. 2006). PirB acts to constrain plasticity, possibly to stabilize neural circuits (Syken et al. 2006). Therefore selective inhibition of PirB could be a possible option to promote plasticity. A second molecule, Lynx 1, has been shown to limit the extent of plasticity in adult visual cortex (Morishita et al. 2010). Lynx1 links to nicotinic acetylcholine receptors to reduce their sensitivity to their ligand (Morishita

et al. 2010). Lynx 1 mRNA and protein levels both increase after the end of the critical period (Morishita et al. 2010). The knock-out mice for Lynx1 showed the same level of OD during the critical period, but OD plasticity was significantly increased in adulthood (Morishita et al. 2010). This suggests that Lynx 1 acts as molecular “brake” to stabilize the circuits and prevent plasticity (Morishita et al. 2010). These findings also underline how neuromodulators, and acetylcholine in particular, are likely to play a fundamental role in regulating cortical adult plasticity.

1.7 Aim of the study

This study aims to investigate whether plasticity of inhibitory neuronal circuits plays an active role in the process of cortical map reorganization in adolescent rats. In particular, I will probe whether innocuous sensory deprivation induced by whisker trimming induces changes in the inhibitory feedback circuits comprising fast-spiking interneurons and pyramidal cells in layer 2/3 deprived cortex.

It is well established that whisker trimming induces cortical map reorganization (Buonomano and Merzenich 1998). The evolution of cortical map reorganization can be imaged with different techniques, including blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI) (Alonso et al. 2007). Previous work in my lab has shown that unilateral passive deflection of the C row of whiskers at 5 Hz in anesthetised rats generates a positive BOLD response in the area corresponding to primary somatosensory cortex of the contralateral hemisphere. When all but the C row of whiskers are trimmed daily for 3 days, the same type of stimulation generates an expanded positive BOLD signal in primary somatosensory cortex, and activation in secondary somatosensory cortex (Alonso et al. 2008). The positive BOLD signal reaches its peak of expansion after 3 days, as after 7 days of deprivation the signal retracts back towards normal levels, but remains expanded. The progression of map expansion is perfectly matched by electrophysiological measures of connectivity between pyramidal

cells of layer 2/3 in deprived cortex (Barnes and Finnerty 2010). After 2 - 3 days of trimming, in fact, the connectivity between layers 2/3 excitatory cells increases from 4% to 12%. This increase in probability of connection is, like the fMRI signal expansion, temporary, and after 7 days of deprivation it returns back to control values. These interesting findings lead to the question of how deprivation affects the inhibitory circuits within the same layer. The increase in the excitatory positive feedback, generated by the rise in connectivity between layer 2/3 pyramidal neurons, could lead to uncontrolled spread of excitation. A reduction of 10% in inhibition in fact is sufficient to generate epileptic activity (Chagnac-Amitai and Connors 1989). Therefore, it is extremely important to investigate whether there is a change in the balance between excitation and inhibition, and, if so, which plasticity mechanisms are involved. There is already structural evidence that interneuron axonal arborisations in layer 2/3 are altered in deprived cortex by 2 – 3 days of deprivation (Marik et al. 2010). I chose to investigate local negative feedback circuits of layer 2/3 because 80% of the inhibitory input on pyramidal neurons of this layer comes from local interneurons (Dantzker and Callaway 2000). I will specifically focus on fast-spiking interneurons because they have been proved to be directly involved in restoring plasticity in adult visual cortex (Pizzorusso et al. 2002).

Brief sensory deprivation will be administered by trimming either the upper three rows (A - C) or lower two (D - E) rows of whiskers for 2 – 3 days, starting from P30, when somatosensory circuitry is mature. The electrophysiological recordings will be performed always in layer 2/3 of the deprived barrel closer to the junction between spared and deprived cortex, or in the C and D barrels of control (non trimmed) animals.

Figure 1.1A shows a schematic of the layer 2/3 disynaptic negative feedback circuit, which is the subject of my investigations. I will consider whether there are changes in these three different aspects of the circuit:

- Intrinsic excitability of FS interneurons, which will be investigated by looking at passive membrane properties, action potential parameters and action potential firing;

- Excitatory input onto FS interneurons, which will be initially assessed initially by recording miniature EPSP in fast-spiking interneurons and subsequently by recording unitary EPSP from pairs of synaptically connected FS interneurons and pyramidal cells;

- Inhibitory input on pyramidal neurons, which will be probed by recording both miniature IPSC from pyramidal cells and uIPSP in pairs of synaptically connected FS interneurons and pyramidal cells.

The results of these experiments will clarify whether plasticity of local feedback inhibitory circuits in mature neocortex is required for cortical map reorganization. It will also be important to compare these results with the changes found within the excitatory circuits, to establish if they are complementary or antagonistic.

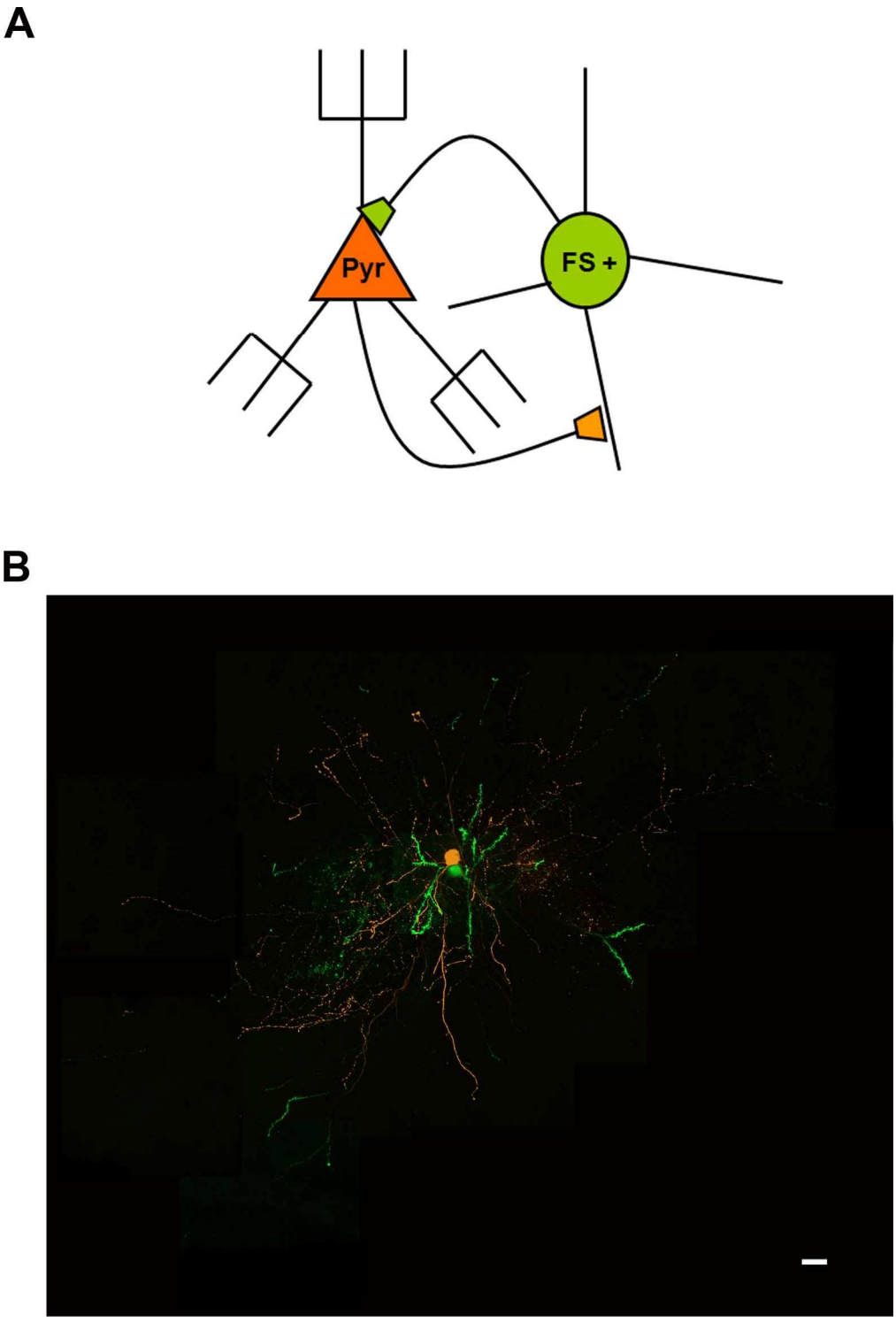

Figure 1.1 Disynaptic negative feedback circuit

A. *Schematic of a disynaptic feedback circuit comprising a FS interneuron (green) and a pyramidal cell (orange) in layer 2/3 of primary somatosensory cortex. The pyramidal cell makes an excitatory connection onto the FS interneuron, which in turn inhibit the pyramidal*

cell through an inhibitory connection. ***B****. Confocal reconstruction of a pair of synaptically connected FS interneuron (orange) and pyramidal cell (green).Scale bar: 20 µm.*

Chapter 2 Methods

2.1 Animal housing and welfare

All procedures were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986. All Sprangue Dawley rats were obtained from Charles River Laboratories UK (Margate, UK). Animals were housed in cages, singularly or in groups, with food and water *ad libitum,* under a 12 hour dark-light cycle. The animals used for this thesis were all males.

2.2 Trimming protocol

Rodents have specialised hair located on their snout, called whiskers or vibrissae. The rodent's whiskers function as tactile organs. The vibrissae are located on the snout of the rats in five rows (A - E, dorsal to ventral) of four to six whiskers each (1 - 4 arcs, caudal to rostral) plus four more caudal longer, straddler whiskers (α, β, γ and δ) sited between the rows.

Sensory deprivation was induced by trimming bilaterally either the 3 dorsal rows (A - C) plus the straddler whiskers α, β and γ or the 2 ventral rows (D - E) plus β and γ (Figure 2.1). Trimming was performed daily (including weekends) using small surgical scissors. Animals were restrained during the procedure by enwrapping them in a soft piece of fabric. The scissors were gently leant against the skin and whiskers cut at the base, placing attention to not damage the follicles. Sham animals where used as controls. Sham trimming involved gently rubbing the scissors against the skin of the snout every day to mimic the trimming procedure, but no whiskers were cut. Rats which presented whiskers abnormalities were not studied if extensive damage was present.

Trimming started at postnatal day 30 (P30), when the corresponding maps in primary somatosensory cortex had already been established (Stern et al. 2001) and was performed for 2 or 3 days. The trimming protocols were chosen to generate a junction

between spared and deprived cortex that was located in both protocols between the C and D barrels.

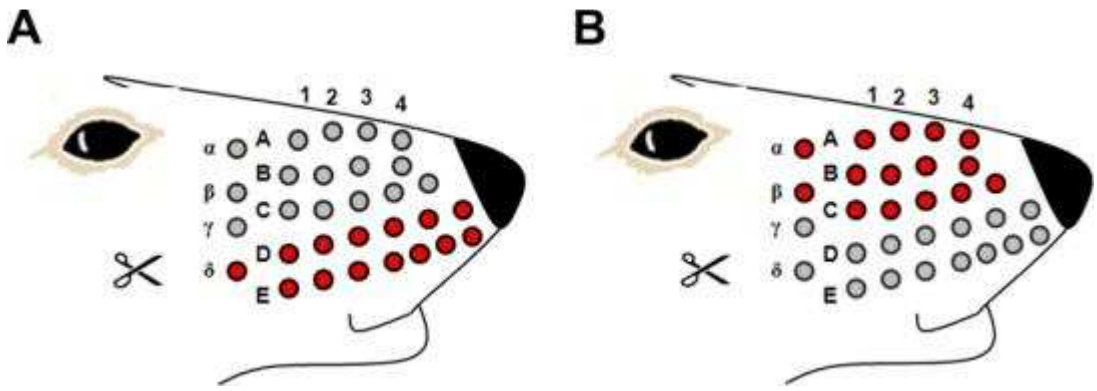

Figure 2.1Trimming schemes

A*. Schematic representing the A - C trim on the right side of the rat's snout. The whiskers, shown as circles, are arranged in 5 rows plus the 4 outliers α – δ. The red circles represent spared whiskers, while the gray circles represent trimmed whiskers.* ***B****. Schematic as in A, but showing a D – E trim.*

2.3 Dissection and brain slice preparation

Rats were moved from their home cage to a small chamber where they were anesthetized with 3% Isofluorane ((Isoflurane-Vet, Merial Animal Health Ltd, Essex) in 1 L/min O_2. After checking the absence of reflex response to tail pinching, a midline incision was made and the heart and the neck veins were exposed. The neck veins were cut and the descending aorta was clumped. The tip of the perfusion tube was then introduced into the left ventricle. The animals were perfused with 35 ml of ice-cold dissection artificial cerebrospinal fluid (ACSF). The brain was dissected from the skull and kept in iced-cold ACSF bubbled with 95%O_2/5%CO_2. The cerebral hemispheres were separated along the midline and each hemisphere was glued to the microtome block on the side of the cut. 350 µm thick slices were cut with an inclination of 65° to the midline using a vibraslice (Campden Instruments)

(Finnerty et al. 1999). The slices were kept in an incubation bath containing bath - ACSF constantly bubbled with 95%O_2/5%CO_2 at 34 - 36˚C and allowed to recover for at least one hour before starting to record.

The dissection ACSF contained the following: (in mM): 108 choline-Cl, 3 KCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 10 Dextrose and 3 $NaC_3H_3O_3$ (sodium pyruvate), 2 $CaCl_2$, 1 $MgCl_2$. The bath ACSF solution included (in mM): 120 NaCl, 3 KCl, 23 $NaHCO_3$, 1.25 NaH_2PO_4, 10 Dextrose, 2 $CaCl_2$, 1 $MgCl_2$. All solutions were maintained at 285 mOsm during dissection, incubation and recording. The standard internal solution for the patching pipette during current-clamp recording contained (in mM): 130 KCH_3SO_4, 8 NaCl, 2 KH_2PO_4, 2 Dextrose, 10 HEPES, 4 MgATP, 0.3 GTP, 0.5 ADP K Salt, 8 Biocytin and either 1 Alexa Fluor (AF) 488 or 1 AF 568 (Invitrogen, UK). The tip of the pipette was loaded with internal solution that did not contain any dye in order to avoid leaks of dye during patching and thereby, increase the quality of confocal images. The internal solution used for voltage clamp was similar to the one used during current-clamp recording with the exceptions that: 130 mM potassium methylsulphate (KCH_3SO_4) was substituted with 130 mM cesium methanesulfonate (CH_3O_3SCs); and 10 mM lidocaine bromide (QX314) was added to the solution. Internal solutions had osmolality of 285 mOsm and were adjusted at pH 7.3 using KOH and CsOH respectively.

2.4 Electrophysiology

Brain slices were transferred to the immersion chamber and visualized using an Olympus BX51W1 light microscope (Olympus UK Ltd). The whisker barrels were identified using a 2X objective with bright field transillumination (Figure 2.2A); a grating was used to hold the slices in position. The chamber was submerged with oxygenated bath - ACSF at 37˚ C and flowing at 3 ml/min. Neurons were visualized using a 40X water objective and infrared differential contrast video (IR-DIC) microscopy. The real time images were acquired with

the digital camera SPOT Insight QE (Diagnostic Intruments, MI, USA) and visualized on-line using the SPOT interface program software (Diagnostic Intruments, MI, USA).

Recording pipettes were pulled from borosilicate glass capillaries (1.5 mm external diameter, 0.86 mm internal diameter, Harvard Apparatus Ltd., Endenbridge, UK) using a Flaming/Brown micropipette puller (Model P97, Sutter Instruments Ltd., Novato, CA) and had a 5 - 10 MΩ resistance. The pipette was gently introduced into the slice with a small positive pressure to gently blow away tissue surrounding the neuron to be patched. Once the pipette was close enough to the cell membrane to form a clear dimple, the pressure was released to form a stable seal of resistance ≥1 GΩ and a gentle suction was applied to break into the cell.

2.4.1 Cell identification

The cells were first selected based on morphological characteristics. Their identity was then confirmed electrophysiologically by analyzing the firing response to 500 ms pulses of depolarizing currents (0.4 – 1.0 nA).

The morphological identification of pyramidal cells was usually quite easy and successful. In a subset of experiments, drugs were already applied in the bath or in the internal solution (voltage clamp experiments). Since in these cases the electrophysiological characterization of neural firing was not possible, the cells were accepted as pyramidal if they presented; a triangular soma shape, a clear apical dendrite pointing toward the pia and spiny dendrites. In contrast, the differences between fast-spiking interneurons and non fast-spiking interneurons were often minimal and the IR-DIC images can be misleading. A further electrophysiological characterization was always required.

At the end of the recording, enough dye had entered the cells and diffused into the axon and the dendritic arborisations to be visualized with epifluorescence. The pipette was then

gently pulled away leaving the neuron intact. Epifluorescence and bright field (BF) images were collected with a 5X objective to locate the cell in the slice (Figure 2.2B). The precise cell location was obtained by merging a 5X bright field picture of the slice taken at the end of the recording and a 5X epifluorecence picture of the filled cell using the merge function of SPOT interface program software (Diagnostic Intruments, MI, USA).

It has to be noted that FS interneurons comprise both basket cells and Chandelier cells. Based on the electrophysiological characterization only, it was not possible to distinguish between these two cell types. A subset of 10 interneurons was imaged after recording; none of these neurons presented the morphological characteristics of Chandelier cells. It is however impossible to exclude that a minor portion of Chandelier cells were recorded and included in the FS interneuron group.

2.4.2 Slice fixation

After recording, the slice was fixed in 2% paraformaldeide (PFA), 0.5% gluteraldeide in 1% phosphate buffer solution (PBS) for 30 minutes at 4˚C. The tissue was then washed in 1% PBS for 6 times to remove any residue of PFA and mounted on glass cover slides (Superfrost, Matsunami Glass Industries, Osaka, Japan) with Fluoramount, an aqueous mounting medium (Sigma-Aldrich, Dorset, UK) and covered with glass cover slips. The coverslips were sealed to the glass using nail varnish.

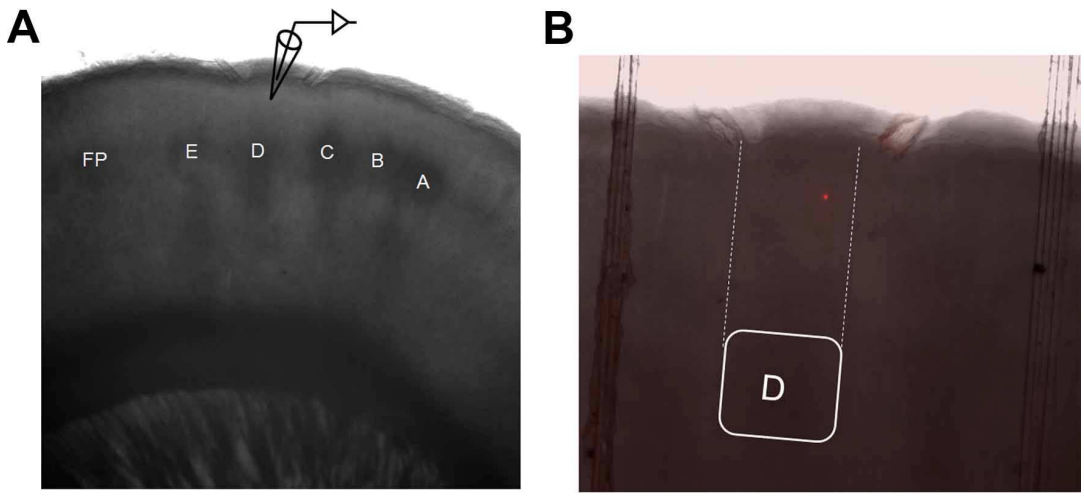

Figure 2.2 Confirming location of recorded neurons

***A**. 2X bright field image of a brain slice. Dark areas correspond to whisker barrel rows, named A – E. The forepaw representation is labelled FP. **B**. 5X image obtained merging a 5X BF image of the slice in A and a 5X epifluorescent image of the recorded interneuron, filled with AF568. The rectangle delimitates the D barrel in layer 4; the dotted lines delimitate the corresponding barrel column in the supragranular layers.*

2.4.3 Current-clamp recordings

The whole-cell current-clamp technique measures the cell membrane potential. This is achieved by connecting a pipette to a unity gain pre-amplifier with high input resistance (much higher than the cell and pipette resistance). When a current source is connected to the amplifier, current can be injected through the pipette into the cell to induce depolarization or hyperpolarisation of the membrane potential.

2.4.3.1 Series resistance compensation

When current flows through the micropipette, a potential drop occurs across the micropipette tip. Hence, the voltage recorded is not the true membrane voltage but the summation of the membrane voltage plus the voltage dropped across the pipette. To overcome this problem and record the real membrane potential, it is necessary to

introduce a bridge balance circuit. The bridge balance circuit consists of a differential amplifier that subtracts a scaled current (scaled to the microelectrode resistance) from the voltage recorded at the back of the micropipette. Series resistance was compensated immediately after breaking into the cell and constantly monitored and adjusted during recordings. Cells were accepted for analysis when series resistance compensation was less than 40 MΩ.

2.4.3.2 Capacitance compensation

A further problem during recording in current-clamp mode is generated by the pre-amplifier input capacitance. This input capacitance comes from various sources as; the capacitance across the glass wall of the portion of the micropipette immersed in the bath, the capacitance from the rest of the micropipette to nearby grounded surfaces; the capacitance from the micropipette holder; and the capacitance to ground at the input of the buffer operational amplifier (Axon Guide, 1998). This input capacitance combined with the resistance of the microelectrode acts as a low pass filter. This causes high frequency signals to be lost. In order to minimize the filtering of the input signal it is possible to take some measures to reduce the physical magnitude of the different sources, e.g. by reducing the level of fluid in the bath as possible, or by thickening the walls of the micropipette. However, these measures are not always sufficient to significantly reduce the overall input capacitance and further electronic compensation is required. Capacitance compensation, also known as negative capacitance, is obtained by injecting a current (ideally equal to the current that flows through the capacitance) through an amplifier placed at the output of the unity gain pre-amplifier. Capacitance compensation was carefully adjusted immediately after break in the cell. Exciding in capacitance compensation (over 7 pF) resulted in signal oscillations and eventually cell death. To optimize capacitance compensation and bridge balance, 10 mV current steps at 50 Hz were applied to the cell. Both series resistance and capacitance were constantly monitored and adjusted before data traces were acquired.

2.4.3.3 Intrinsic excitability

After adopting the whole-cell configuration and adjusting series resistance and capacitance compensation, the identity of the cell and its intrinsic excitability was assessed by inducing action potential firing through 500 ms pulses of 0.4, 0.6, 0.8, and 1 nA current injections (Figure 2.3D). The cells were held at their resting membrane potentials. The pulses were repeated in sequence at least twice for each cell. Then, smaller currents were injected to identify the rheobase, the minimum amount of current required to induce an action potential. The frequency of action potentials was plotted against the amount of current injected and a regression line was fitted to the points. The slope and the intercept of the regression lines were then used to quantify intrinsic excitability.

The action potential shape was also analyzed using the following parameters (Figure 2.3C):

- Action potential threshold, which was determined as the point of the action potential where the increase in membrane potential was faster than 20 V/s;
- Action potential amplitude, which was determined as the voltage difference between peak and threshold;
- Action potential half-width, which measured the width of the action potential at half of the peak amplitude;
- After hyperpolarization (AHP), this was measured from the threshold to the most hyperpolarized potential after the action potential peak.

The parameters were extrapolated from the data using the custom written Labview (National Instruments, Austin, TX, USA) program 'GTF AP detector 1.4'.

2.4.3.4 Passive membrane properties

The cell membrane has passive properties: membrane potential (V_m), membrane resistance (R_m), membrane capacitance (C_m) and membrane time constant (τ_m). To

measure passive membrane properties, a hyperpolarizing 100 ms pulse of -0.1 nA was applied at the end of the 500 ms current injections. Ohm's law was used to extract the membrane resistance from the voltage change generated by the -0.1 nA current injection:

$$R_m = \Delta V / I$$

The membrane time constant, which is defined by the time taken to reach 1/ (1-e) (~ 63 %) of the maximum change in voltage, was calculated from the exponential decay of the curve fitted to the data. The membrane capacitance was calculated as the quotient of the membrane time constant and resistance:

$$C_m = \tau_m / R_m$$

The analysis was performed using the custom written Labview (National Instruments, Austin, TX, USA) program 'GTF passive membrane properties'. The measures were acquired averaging 3 - 4 traces for each cell.

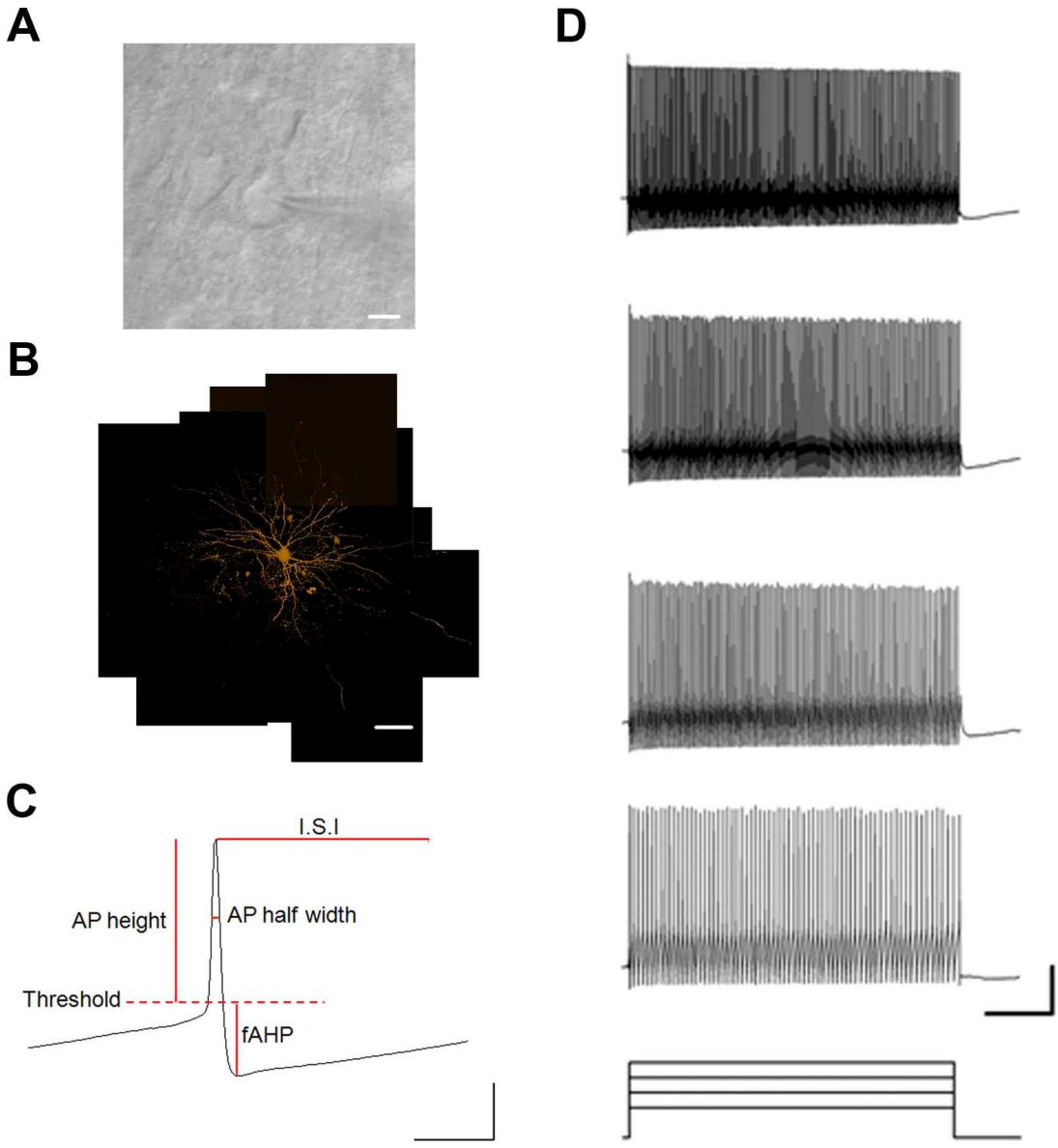

Figure 2.3 Morphological and electrophysiological characterization of a FS interneuron

***A**. IR-DIC image of a FS interneuron patched in whole – cell mode. The interneuron is surrounded by two cells with clear pyramidal cell morphology. Scale bar: 10 µm. **B**. Confocal reconstruction of the FS interneuron in A. Scale bar: 50 µm. **C**. Action potential fired by the cell in A and B. The schematic reports the parameters used to characterise the AP shape: threshold, AP height, AP half-width, fast after-hyperpolarisation amplitude (fAHP) and inter-spike interval (ISI) (from peak to peak).Scale bars: 2 ms, 20 mV. **D**. AP*

firing patterns in response to 500 ms current injections of respectively (from bottom to top): 0.4, 0.6, 0.8 and 1.0 nA. Scale bar: 100 ms, 30 mV.

2.4.3.5 Miniature excitatory postsynaptic potentials (mEPSPs)

In absence of presynaptic activity or stimulation, the resting membrane potential of a cell shows small variations. Those depolarisations are thought to be due to the release of single vesicle of neurotransmitter, which occurs stochastically as consequence of the probabilistic nature of the release process (Fatt and Katz 1952). mEPSPs can then be considered as a general non-specific measure of the excitatory drive onto a cell and changes in their amplitude or frequency can be used as general indicators of circuit alterations (Cheetham et al. 2007).

mEPSPs were recorded from pyramidal cells and interneurons in current-clamp mode. The cells were held at resting membrane potential and the depolarisations induced by the activation of AMPA and NMDA receptors by glutamate were recorded as positive peaks. Spontaneous EPSPs (sEPSPs) were recorded immediately after the characterization of the cell firing pattern.

In order to record mEPSPs, 1 µM tetrodotoxin (TTX), a reversible voltage gated sodium channel blocker, was applied to the slice to abolish action potential firing. 100 µM picrotoxin (PTX) was also added to the slice in order to block $GABA_A$ receptors and the consequent membrane potential hyperpolarizations, although the cell resting membrane potential is very close to the reversal potential for chloride ions. mEPSPs were recorded in whole-cell current-clamp mode at resting membrane potential in 10 traces of 5 seconds each, 15 minutes after the application of TTX and PTX. A pulse of 0.8 nA was applied just before data collection, to verify that the cell could not generate action potentials. Traces were acquired using an 8-pole Bessel filter with a -3dB corner frequency of 3 kHz, to remove some of the noise while maintaining the physiological signals in current-clamp.

2.4.3.6 mEPSPs analysis

mEPSPs were analyzed using the custom written program GTFspontaneousEPSPs v1.1 by examiner blind to the trim condition. The traces were looked through manually in 100 ms windows to select the events that met the criteria to be considered mEPSPs. Events were detected with two cursors, one to detect the raise of the event and the second to locate the peak. The value reported by the cursor was the average of 1 ms time window around it, in order to get a more accurate value. The criteria used to detect the events were similar to Clements and Bekkers (Clements and Bekkers 1997):

- The peak had to be asymmetric, with a decay time much longer then the rise time,
- The time to peak had to be shorter than 5 ms,
- The amplitude of the peak had to exceed 2.5 times the root mean square of the noise, which was averaged from a subset of different traces.

Some of the events occurred simultaneously and this made it difficult to identify the baseline of the second events. Although some of the analysis reported in the literature ignored overlapping events (Simkus and Stricker 2002), in this thesis events were considered as different when the time between the peak of the first event and the start of the second one was bigger than 1 ms, as previously reported by Cheetham et al. (Cheetham et al. 2007). 50 subsequent events from a single trace were analyzed for each cell and average mEPSPs frequency and amplitude were calculated for each cell.

2.4.4 Voltage clamp recording

The voltage clamp technique consists in recording the current injected into the cell to keep the membrane potential set to a constant value. Although this process does not simulate any natural event, it is very useful for different reasons; capacitive currents are less of a problem compared to current-clamp since they occur only at the application of a different voltage step; the currents recorded are proportional to the membrane conductance (determined by the fraction of channels open) and the holding voltage can be changed to control the open probability of voltage gated ion channels (Axon guide).

In order to clamp the voltage of the cell, it was also necessary to block the leakage currents without compromising the currents required for the cell to survive. The internal solution used during voltage clamp recordings was then modified compared to the one used for current-clamp recordings, as follows: 130 mM potassium methylsulphate (KCH_3SO_4), that was substituted by 130 mM cesium methanesulfonate (CH_3O_3SCs) and 10 mM lidocaine bromide (QX314) that was added to the solution. The caesium ions block the potassium channels (Adelman and Senft 1966) while the QX314 applied internally blocks the voltage gated sodium channels (Strichartz 1973), preventing the cell from firing action potentials. Those measures together increased the membrane resistance of the cell, implementing the quality of the voltage clamp. However, it was very difficult to increase membrane resistance above 200 MOhm, even 30 minutes after the diffusion of caesium ions and QX314 into the cell.

2.4.4.1 Voltage and Space-clamp limitation

The voltage clamp technique has been extensively used to investigate synaptic physiology in the past three decades. Although extremely useful, this technique has also a few important limitations, which need to be taken into consideration.

A first limitation comes from the complex electronic structure of neurons, which affects the measure of dendritic currents (Spruston et al. 1993). Ideally, in voltage clamp mode, the membrane potential should be fixed at a specific value throughout the whole neuron. However, in practice, the current injected by the electrode at the soma cannot control the membrane potential in distal dendritic arborisations. Moreover, the resistance and the capacitance of the neural membrane generate a filter, which distorts the shape of distal events (Spruston et al. 1993, Williams and Mitchell 2008). The distortion is distance-dependent, with the somatic voltage clamp recovering only 50% of the amplitude of events

artificially generated just 90 µm from the soma in a pyramidal cell of layer 5 (Williams and Mitchell 2008).

A further limitation in recording in voltage-clamp mode comes from problems in series resistance compensation. As for current-clamp recording, in fact, the voltage recorded at the top of the pipette is different from the voltage of the cell because of the voltage drop across the tip of the pipette. The series resistance generates significant voltage errors and, although it is possible to compensate for these errors, the compensation is never perfect.

In order to minimize electronically the effect of pipette resistance, a signal proportional to the measured current was used to increase the command voltage. This system, known as resistance correction, has some limitations. A 100% correction would often kill the cell, as the circuit becomes unstable and starts to oscillate. Oscillations of the signal were reported even at lower percentage of compensation, and the maximum resistance compensation achieved in pyramidal cells was 70%. Pipette capacitance was compensated in voltage clamp recording, to remove the spike generated at the moment of the current injected to step the potential and to increase the stability of the series resistance compensation. The capacitance of the cell was also compensated, to remove the transient required to charge the cell membrane. This was done mainly to avoid saturation of the system during series resistance compensation, since no meaningful data can be acquired during the transient. To adequately compensate resistance and capacitance, 10 pA pulses of current were injected into the cell with a 10 Hz frequency.

2.4.4.2 Miniature inhibitory postsynaptic currents (mIPSCs)

Voltage-clamp experiments were performed in whole cell mode to record miniature inhibitory postsynaptic currents (mIPSCs). Inhibitory currents are generated in the neocortex by the release of GABA and the subsequent activation of $GABA_A$ receptors in

the postsynaptic terminal. The Cl^- ions flux through the channels is responsible for the hyperpolarization of the cell, based on the driving force determined by the difference in concentration between the intracellular and extracellular space. Miniature events are recorded in absence of AP firing and are tough to be generated by the release of single vesicles of neurotransmitter. To record mIPSCs the membrane potential was clamped at 0 mV. This was necessary because the resting membrane potential is too close to the reversal potential for chloride ions to properly isolate the currents from the background noise of the recording.

Pyramidal cells in L2/3 of barrel cortex were patched with the voltage-clamp internal solution containing caesium ions and QX-314. Membrane potential was recorded immediately after break in, since after that the cells started to depolarize quickly, as a result of the leak current blockage. Current injections were able to induce action potentials in the first few minutes, but their shapes were severely altered (they were much broader and elongated). This made very difficult to assess electrophysiologically the type of cell that was recorded; cell identification was therefore based on morphological characteristics and post-hoc analysis of epifluorecence images or confocal reconstructions (see section 2.4.1). Membrane resistance was assessed by injecting a -0.1 nA hyperpolarizing current in current-clamp. Cells were considered suitable for voltage-clamp when: 500 ms pulse of 0.4 nA current injection did not induce any action potential or any further depolarization away from the steady state membrane potential; the membrane resistance was greater than 140 MΩ; and the series resistance was lower than 35 MΩ.

The cell was initially clamped at resting Vm; series resistance and capacitance compensation were adjusted as necessary. The membrane potential was then gradually shifted to 0 mV. In order to acquire mIPSCs, 1 µM tetrodotoxin (TTX), a reversible voltage gated sodium channel blocker, was applied to the slice to abolish action potential firing. 50 µM (2*R*)-amino-5-phosphonovaleric acid (APV) and 20 µM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were also added to the slice, in order to block glutamate receptors AMPA

and NMDA, although at holding potential of 0 mV, the cationic glutamatergic currents should be zero. 500 ms pulses of 0.4 nA and -0.1 nA were applied in current clamp mode before switching to voltage-clamp mode to verify that the cell could not generate action potentials and the membrane and series resistances were satisfying the criteria to proceed. mIPSCs were recorded in whole-cell voltage-clamp mode at the holding potential of 0 mV in 5 traces of 10 seconds each, at least 15 minutes after the application of TTX, CNQX and APV and 30 minutes after breaking in, in order to maximize the effect of cesium ions and QX314. Traces were acquired using an 8-pole Bessel filter with a -3dB corner frequency of 2 kHz, to remove some of the noise while maintaining the physiological signals. After data collection, the cell was moved in current-clamp mode to check the final membrane potential and resistance. In a subset of experiments, TTX, CNQX and APV were added to the slice when placed in the recording chamber and constantly washed on the slice. mIPSCs were still recorded 30 minutes after break in, to maximize the effects of Caesium ions and QX314 .

2.4.4.3 mIPSCs analysis

mIPSCs were analyzed using the custom written program GTFspontaneousEPSPs v1.1 by examiner blind to the trim condition. The analysis was performed as per mEPSPs, although traces were looked through manually in 200 ms windows because the frequency of these events was much lower and it resulted easier to discriminate the event from the baseline noise. The criteria used to detect mIPSCs were the same as per mEPSPs. Because of the frequency of mIPSCs were lower compared to the frequency of mEPSPs, a 10 s trace was analyzed for each cell. Average frequency and amplitude were then calculated for each cells and reported in cumulative fraction plot.

2.4.5 Recordings of synaptically connected pairs of neurons

Recordings started establishing a whole cell patch onto a putative FS interneuron. Once the identity of the cell was confirmed by its firing patterns in response to 500 ms depolarizing current pulses, a second pipette was introduced into the slice. The first

neuron was usually filled with K+ based internal solution containing AF 488 while the second pipette would have been loaded with K+ based internal solution containing a different dye (AF 568). Since connectivity between pyramidal cells and parvalbumin positive interneurons has been showed to be inversely correlated with their intersomatic distance in layer 2/3 of somatosensory cortex (Packer and Yuste 2011), the second pipette was addressed to patch the closer pyramidal cell, identified by morphological characteristics. Once the second whole-cell patch was established and the identity of the cell was confirmed electrophysiologically, the connectivity was tested. To test the presence of an excitatory connection, the pyramidal cell was induced to fire action potentials by injecting high currents (1.5- 2.5 nA) in 8 pulses of 2 ms each at 20 Hz. 10 traces of 8 pulses each were acquired consecutively, with 5 or 10 seconds between each trace, with an 8-pole Bessel filter of 10 kHz. The amount of current injected was established choosing the minimum amount of current that could reliably elicit one action potential for each pulse. The interneuron was recorded in current-clamp at resting membrane potential. If the pyramidal cell was connected to it, unitary excitatory postsynaptic potentials (uEPSPs) would be generated in the interneuron and appear as a short latency positive peaks in response to the action potentials elicited in the presynaptic neurons. Traces of the action potentials elicited in the presynaptic cell were visualized as raw traces, to check that one action potential was elicited for each pulse of current injected; traces of the postsynaptic cells were visualized as averaged, in order to facilitate the detection of small connections and minimize the noise to signal ratio. At least 50 traces were collected at action potentials frequencies of 20, 40 and 10 Hz in the specified order. Series resistance and capacitance compensation were checked and adjusted for both cells after the acquisition of each set of traces. At the end of the data acquisition, the cells were tested for the presence of a reciprocal connection. In order to identify uIPSPs the pyramidal cell was hold at -50 - -55 mV while the same protocol for inducing action potentials was applied to the interneuron. Once the connection was confirmed, 50 traces at 10 or 20 Hz were acquired in sets of 10 each. uIPSPs were acquired with an 8-pole Bessel filter of 3 kHz because of their smaller and slower nature compared to uEPSPs. At the end of the recordings, the pipettes were gently withdrawn from both cells and 5X images in bright field and epiflurescence were

acquired to localize the cells within the barrel. The slice was fixed (section 2.4.2) and imaged with confocal microscopy in the following 24 hours.

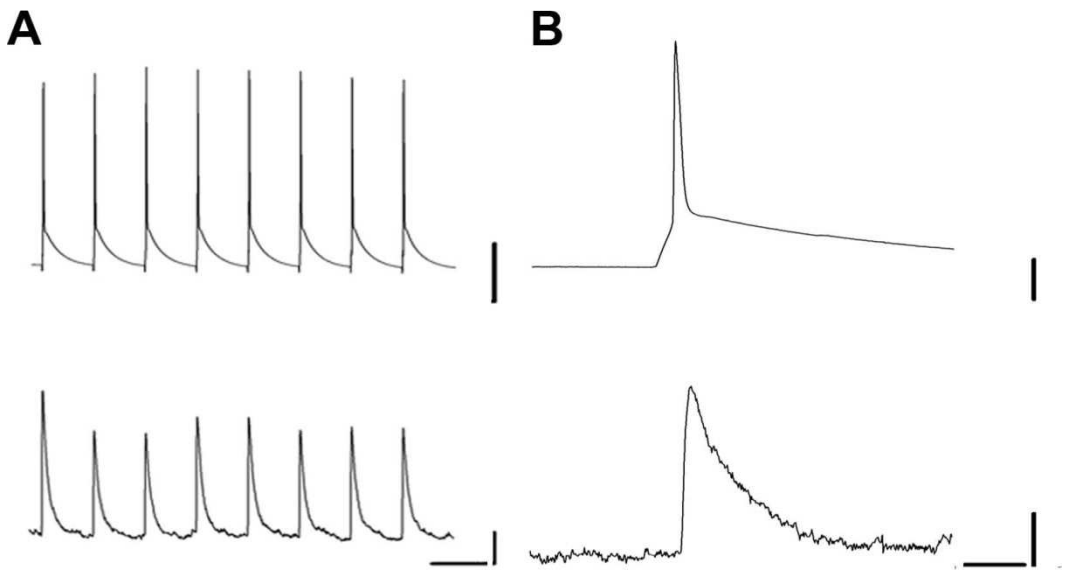

Figure 2.4 Unitary excitatory postsynaptic potentials (uEPSP)

A**. 8 current pulses were injected into a presynaptic pyramidal neuron to fire 8 action potentials at 20 Hz. The action potential induced 8 short-latency uEPSPs in the postsynaptic FS interneuron (average of 50 traces). Scale bars (from top to bottom): 30 mV, 1 mV, 50 ms.* ***B. *Expanded section showing a single action potential in the presynaptic pyramidal cell and a single $uEPSP_1$ trace in the FS interneuron. Scale bars (from top to bottom): 20 mV, 1 mV, 5 ms.*

2.5 Confocal reconstruction of dyed-filled neurons

Dye filled neurons were imaged using a LSM510 META laser-scanning confocal microscope (Carl Zeiss Ltd., Oberkochen, Germany). AF568 was excited using the 543 nm line of a helium-neon laser (laser power ~80 % of 1 mW, detector gain 700 – 720). The light pathway consisted of HFTUV/488/568/633 primary dichroic filter, a secondary

NFT545 filter and a long pass emission filter set at 560 nm. AF488 was excited using the 488 line of an Argon laser (laser power ~5 % of 30 mW, detector gain 700 -730). The light path consisted of a HFT488 primary dichroic filter, a NFT490 secondary dichroic filter and the emitted light was band pass filtered at 500 – 550 nm.

A first confocal Z - stacks of the cell(s) was acquired with a 20X magnification, 0.5 NA Plan Neofluar air objective to visualize the cell body (bodies) and most of its (their) dendritic arbour(s). Scans consisted of 1024 x 1024 pixels, scan zoom 1, pixel time 1.28 µs. The pinhole diameter was set to 0.88 Airy Units (AU) for AF568 and to 1 AU for AF488 such that both channels had the same optical slice thickness of 2.27 µm. The amplifier offset was used to optimise the signal to noise ratio and was typically set at -0.05, the amplifier gain was always 1.

Medium and high resolution images were acquired with a 63X magnification 1.2 NA C-Apochromat water immersion objective with coverslip correction. The thickness of the coverslip (140 - 180 nm) used to cover the top of the fixed slice was measured with an electronic micrometer (RS Components Ltd., Corby, UK) and used to manually adjust the lens. To maximize the performance of the confocal microscope, the position of the pinhole and the collimator were adjusted each time using 0.1 – 4.0 µm Tetraspeck fluorescent beads (Invitrogen). Beads were mounted in Fluoramount and stored at 4 ℃. A bead was selected and imaged with the 63X water immersion objective with zoom 4 and the position of the collimator and the pinhole were adjusted to optimize the excitation and collection of light emission for each fluorophore. Scans consisted of 1024 x 1024 pixels, scan zoom 1, pixel time 1.28 µs, optical slice thickness 0.43 µm for both channels. Medium resolution images were acquired starting from the cells body (bodies) to further extend to the surrounding axonal and dendritic trees.

High resolution images of putative synaptic contacts were acquitted with the 63X water immersion objective, 4X zoom. The optical slice thickness was changed to 0.2 µm, pixel

time 1.60 µs, frame size adjusted to 512 x 512 pixels, with each imaged averaged 8 times to give a final voxel dimension of 0.07 x 0.07 x 0.2 µm. Subsequent blind adaptive point spread function (PSF) deconvolution (AutoDeblur, Media Cybernetics, Inc., Silver Spring, MD) was performed on the high resolution images acquired.

2.6 Statistics

All statistical tests were carried out in SigmaPlot (11.0) (Systat Software Inc. London, UK), unless stated otherwise. Normally distributed data were described by the mean ± standard error (S.E.M.) and parametric statistical tests were used. If the data were not normally distributed, they were described by the median (interquartile range - IQR) and non-parametric tests were applied. Student's t-tests and Mann-Whitney rank sum tests were used to compare two sets of data. The paired t-test and the Wilcoxon signed rank test were used for pair-wise comparisons of parametric and non-parametric data respectively. Relationships between data sets were tested either with Pearson product moment correlation (for normal data) or with Spearmans rank order correlation (for non-normal data). For longitudinal data, I performed statistical modeling under the generalized estimating equations (Liang and Zeger 1986, Zeger and Liang 1986) using the "stats," "mgcv," "nlme," "MASS," and "gee" packages implemented in R (R Project for Statistical Computing, http://www.r-project.org/). Generalised estimating equations (GEE) with unstructured working correlation matrices were used to account for correlations that are likely to occur in dataset where multiple measurements are taken from single neurons. The two tailed p-values were calculated using the robust (sandwich) estimate of variance.

The models used for AP threshold, AP height, AP half-width and AHP amplitude in Chapter 3 were in the following form:

$$[E(Y)] = \alpha + \beta(\text{RC\_DEP}) + \gamma(\text{RC\_FINAL})$$

Where $E(Y)$ is the expected value of the variable Y (threshold, or AP height, or AP half-width, or AHP amplitude); and α, β and γ are coefficients of the models. The deprivation status of layer 2/3 FS interneurons was modelled with the dummy explanatory variable RC_DEP (deprived = 1, control = 0), while the dummy variable RC_FINAL (final =1, initial = 0) denoted whether the measure was the average of the initial 5 APs or the average of the final 5 APs in the train.

The model used for half-width that included the AHP amplitude and AP amplitude variables was in the form:

$$[E(Y)] = \alpha + \beta(\text{RC\_DEP}) + \gamma(\text{RC\_AHP AMP}) + \delta(\text{RC\_AP AMP})$$

Where $E(Y)$ is the expected value of the variable Y (half-width); and α, β, γ and δ are coefficients of the models. The deprivation status of layer 2/3 FS interneurons was modelled with the dummy explanatory variable RC_DEP (deprived = 1, control = 0) while the effect of AHP amplitude and AP amplitude were modelled respectively by the continuous variables RC_AHP AMP and RC_AP AMP.

Chapter 3 Plasticity of passive and active intrinsic membrane properties of layer 2/3 FS interneurons

3.1 Introduction

In this chapter I investigated the effects of sensory deprivation on the intrinsic properties of layer 2/3 FS interneurons in primary somatosensory cortex. Whisker trimming started between P30 and P43, when the sensory map in layer 2/3 had already been established (Stern et al. 2001) and lasted for 2 or 3 days. Control animals were in the same P32 – P45 age range of deprived animals.

I first looked at the passive membrane properties of FS internurons that include: membrane resting potential (V_m), membrane resistance (R_m) membrane capacitance (C_m) and time constant (τ_m). Active membrane properties were also investigated by comparing action potential (AP) shape parameters between control and deprived conditions. Neuronal excitability was explored by looking at the input-output relationship between the amount of current injected (in whole cell current-clamp mode) and the number of APs fired by the cell.

3.2 Background

The spatio-temporal combination of excitatory and inhibitory drive into a cell determines the probability that a cell fires action potentials. However, the probability of evoking an action potential is also determined by the intrinsic properties of the cell itself. By altering those properties, experience-dependent plasticity and learning and memory can modify neuronal circuits and, at least theoretically, store new memories (Zhang and Linden 2003).

Although only a few studies have investigated the intrinsic excitability of neurons in barrel cortex, plasticity of intrinsic excitability has been extensively studied in other sensory systems, particularly in visual cortex (Desai et al. 1999b, Desai et al. 1999a, Maffei and Turrigiano 2008, Maffei et al. 2006, Maffei et al. 2004). Nonetheless, there is considerable evidence that many mechanisms of plasticity are shared between different cortical areas

(Fox and Wong 2005). Therefore, I will report in these sections studies on plasticity of intrinsic excitability performed in different cortical areas which are relevant to my research.

Early experiments in visual cortical neuron cultures have shown that blocking activity by applying TTX for two days increases the neuronal excitability of excitatory cells (Desai et al. 1999b). The same protocol also increases intrinsic excitability of interneurons, although the effect is smaller, compared to pyramidal cells (Desai et al. 1999a). Interestingly, 2 - 3 days of TTX injection in vivo does not induce a change in intrinsic excitability of excitatory cells in layer 2/3 of the corresponding monocular primary visual cortex (recorded in brain slices), whereas 2 - 3 days of lid suture increases excitability by moving the AP threshold to more hyperpolarized potentials, without affecting the passive membrane properties of the cells (Maffei and Turrigiano 2008). Although the changes in intrinsic excitability I reported so far develop to counter-balance an external change in activity (and therefore have been associated to homeostatic plasticity), there is also evidence that intrinsic plasticity can be potentiated by increased activity. Intrinsic excitability, in fact, is increased in primary visual cortex by sustained activity; a brief period of high frequency firing in layer V excitatory neurons reduces their AP threshold and generates a leftshift of their input-output functions (Cudmore and Turrigiano 2004).

Changes in intrinsic excitability have also been reported as a result of learning in behavioural paradigms. Trace eyelid conditioning in rabbits induces a rapid but transient increase in intrinsic excitability of CA1 hippocampal pyramidal neurons, related to a reduction in the afterhyperpolarisation phase (AHP) that follows the AP (Disterhoft et al. 1986). A similar increase in excitability of CA1 cells due to reduction in the AHP amplitude has also been reported recently in response to fear trace conditioning (Song et al. 2012). Although the change in intrinsic excitability is transient, the effect could be permissive for the memory formation.

Changes in intrinsic excitability as a result of cortical reorganization have been detected after denervation of the forepaw (Hickmott 2005). Layer 2/3 pyramidal cells in the deprived area showed a decrease in excitability; the AP threshold was increased only transiently, but the amplitude of the fast and medium AHP and the duration of the ISI were increased at every denervation time point tested (up to 28 days) (Hickmott 2005).

Only a few studies have reported experience-dependent modification of intrinsic properties in barrel cortex. Unilateral removal of all but the D1 whiskers for 20 - 24 hours induces decreased excitability of layer 2/3 pyramidal cells in the corresponding spared barrel in primary somatosensory cortex of adult transgenic fosGFP mice (Barth et al. 2004). The decreased excitability is associated with depolarization of the threshold potential and increased spike accommodation (spike frequency adaptation) (Barth et al. 2004). Long periods of whisker trimming started during development (Finnerty et al. 1999, Allen et al. 2003) or during adolescence (P19) (Cheetham et al. 2007) do not affect the intrinsic properties of layer 2/3 pyramidal neurons, although trimming during the critical period delays the maturation of layer 2/3 pyramidal cells firing characteristics (Maravall et al. 2004). Even though a few studies reported changes in intrinsic excitability of excitatory cells, only one study, to my knowledge, found changes in the intrinsic properties of interneurons after whisker trimming (Sun 2009). Removing the D row of whiskers from P7 until P30 alters the firing properties of FS interneurons in layer 4 of the deprived barrel cortex. As result of deprivation, the firing frequency decreases, the firing threshold is more depolarised and the input-output function of FS interneurons in deprived cortex is shifted to the right. Although the passive membrane properties of FS interneurons are also significantly affected, with a decrease in R_m and increase in τ_m, the shift of the input-output function is mainly associated with an increase in voltage-dependent potassium channel current I_A (Sun 2009). Interestingly this change in excitability was selective for FS interneurons cells, as none of the above changes were reported in layer 4 non fast-spiking (NFS) interneurons. To my knowledge, no changes in intrinsic properties of layer 2/3 FS interneurons have been reported in barrel cortex as a result of deprivation. Here, I

investigated whether a short period (2 - 3 days) of whisker trimming affects the intrinsic properties of FS interneurons. Trimming started at P30, when the mature firing characteristics of FS interneurons are already well developed (Goldberg et al. 2011).

3.3 Method summary

Bilateral trimming of the upper 3 rows (A, B and C plus the outlier α, β and γ) or the lower 2 rows (D and E 2 rows plus the outliers γ and δ) of whiskers was performed daily from P30 - P43 animals for either 2 or 3 days. Control animals were handled daily and were exposed to a sham procedure. The day of the experiment animals were anesthetized and perfused with dissection ACSF. Brain slices were cut on a 65° angle to the midline (Finnerty et al. 1999), kept in bubbled ACSF at 36℃ for an hour and subsequently at room temperature until recording. Recordings were performed at physiological temperature (37℃) using a K^+ based internal solution (see Chapter 2). V_m was recorded a few minutes after breaking into the cell. The other passive membrane properties were acquired by applying a -0.1 nA hyperpolarizing pulse. R_m was calculated using Ohm's law; T_m was extrapolated by the exponential curve fitted to the membrane hyperpolarisation and the membrane capacitance was calculated from T_m and R_m. FS interneurons were identified by their firing pattern in response to current injections. 0.4 nA, 0.6 nA, 0.8 nA and 1.0 nA were injected in the reported order after break in. The input-output function for each neuron was obtained by reporting the number of action potentials fired in response to the currents injected. The slope and the intercept of the linear curve fitted to the data were used to compare neuronal excitability across the different trimming conditions. AP shape parameters were analyzed in response to 1.0 nA current injection for all cells. The first 5 APs and the last 5 APs of the train were averaged to give the initial and final AP shape parameters.

3.4 FS interneurons identification

Whole-cell recordings were obtained from somata of FS interneurons located in layer 2/3 of primary somatosensory cortex. Cells were fist identified visually under a 40X objective using IR-DIC microscopy. The border between layer 1 and layer 2/3 is located about 150 µm from the pial surface (Beaulieu 1993) and it was clearly discernible under IR-DIC as a net change in cells density. The cells selected for recording were located in layer 2/3, therefore at a depth comprised between 200 µm and 600 µm, where the border between layer 2/3 and layer 4 is to be found (Beaulieu 1993). However, rarely selected neurons were so close to the layer 4 border, and the average distance from the pial surface was 307 ± 11 µm.

Interneurons were first identified based on morphological characteristics: they had a round or a drop-shape soma (Figure 3.1B), which is different from the triangular shape soma with the apical dendrite pointing toward the pia of pyramidal cells (Figure 3.1A). FS interneurons somas were usually slightly bigger than other interneurons and were often found next to blood vessels. However, morphological characteristics alone were not sufficient to identify FS interneurons. Therefore, their final identity was established by looking at the cells firing patterns. Data were reported if: the resting membrane potential was more hyperpolarized then -58 mV throughout recording and the series resistance was less than 40 MOhm. Once the whole-cell was established, 500 ms depolarizing square pulses were injected to induce the cell to fire. FS interneurons firing characteristics have been previously described (see Chapter 1) and they can be summarised as: high firing frequency (up to 400 Hz); no or very little spike frequency adaptation and a fast, deep monophasic after-hyperpolarisations (AHP) (Beierlein et al. 2003, Connors and Gutnick 1990, Kawaguchi 1995). These characteristics are peculiar to FS interneurons and they were used to distinguish unmistakably those from regular spiking pyramidal cells and other non fast-spiking interneurons (NFS) (Figure 3.1ii).

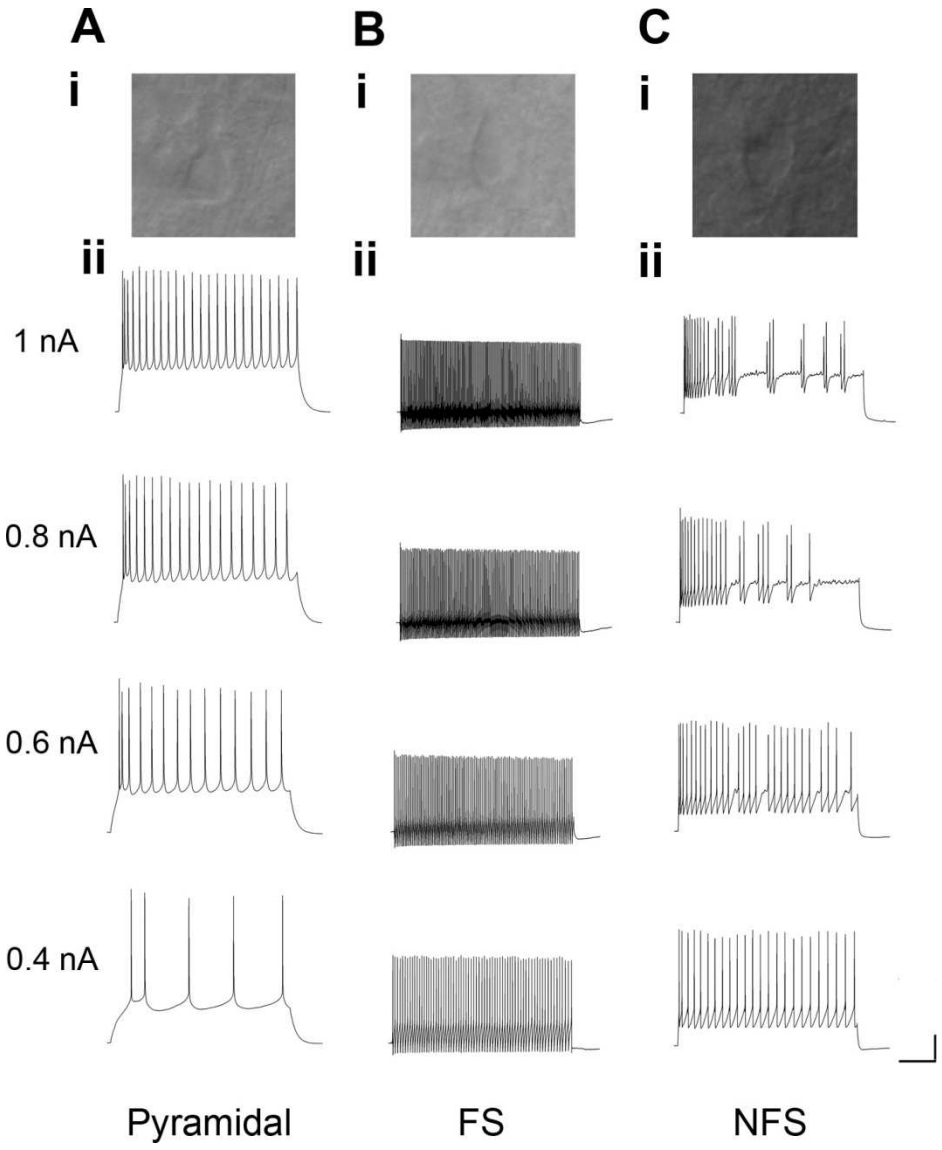

Figure 3.1 Examples of neocortical cells diversity

A. *(i) Soma of a recorded pyramidal cell acquired with a 40X objective using IR-DIC and (ii) AP firing patterns induced by 500 ms square pulses of 0.4, 0.6, 0.8 and 1.0 nA.* ***B****. (i) Example of FS interneuron soma and (ii) firing patterns. Current injections as in A.* ***C****. Example of NFS interneuron morphology and (ii) electrophysiological behaviour. Current injections as above. Scale bars (same for Aii, Bii, Cii): 20 mV, 100 ms.*

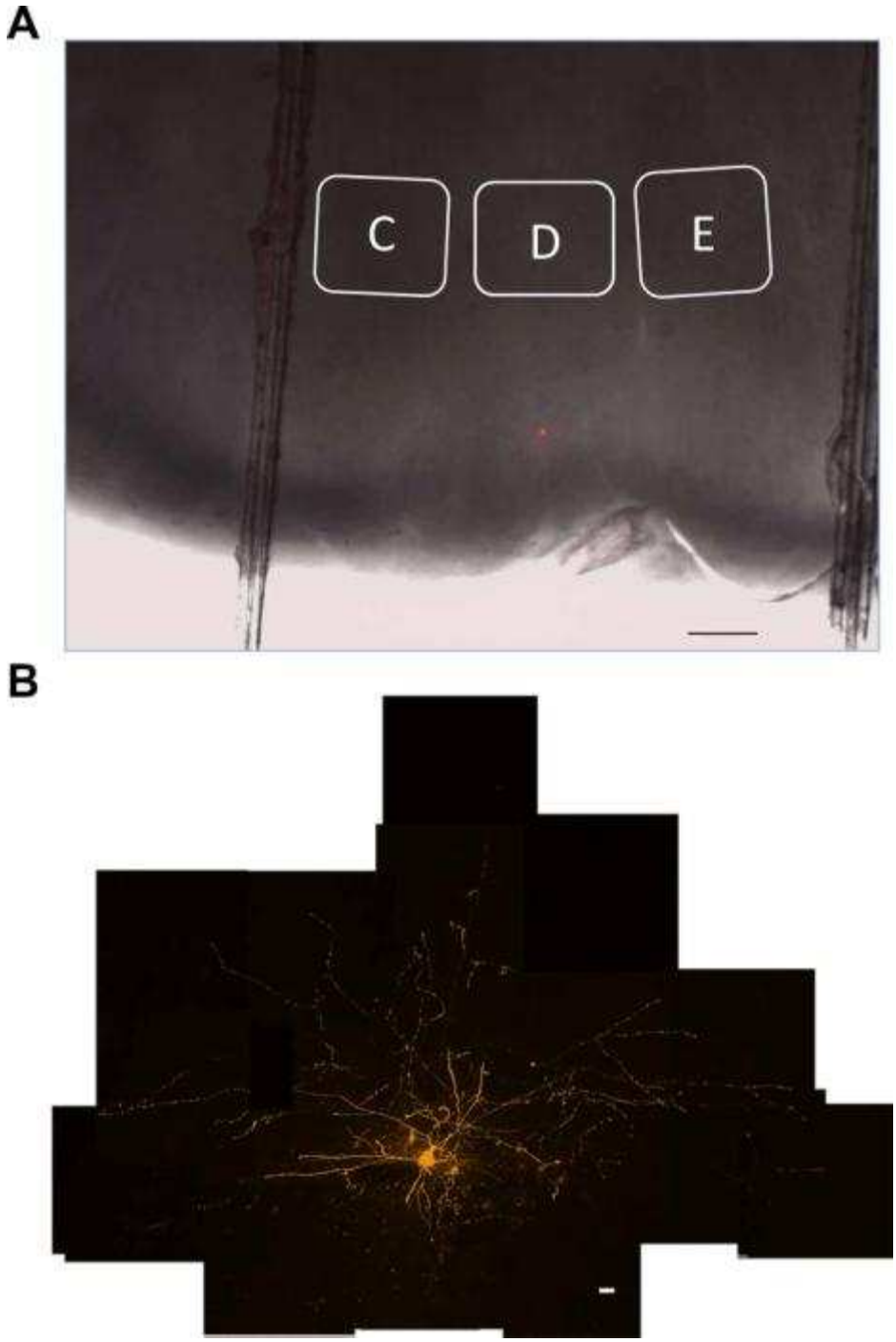

Figure 3.2 Cell location and confocal reconstruction

***A**. Image of a brain slice taken with 5X objective under transmitted light and superimposed to a fluorescent image of the same slice after recording from a FS interneuron filled with AF568 in the D barrel. C, D and E barrels are delimited by white boxes for clarity. Scale*

bar: 200 µm. ***B****. Montage of medium intensity projections from confocal stacks of the same FS interneuron filled with AF568 in A. Scale bar: 10 µm.*

As the internal solution contained fluorescent dye, the exact location of the cell was determined at the end of the recordings by overlapping 5X bright field transillumination images with 5X epifluorescence images of the same portion of slice (Figure 3.2A). A subset of the recorded cells was then imaged using confocal microscopy (Figure 3.2B).

3.5 Passive membrane properties

The membrane passive electric properties affect the way a cell responds to current flowing across the membrane. By changing the passive membrane properties it is therefore possible to affect the neuronal excitability of the cell and the propensity to fire APs. In this section I investigated whether 2 - 3 days of deprivation affected the passive membrane properties of FS interneurons in layer 2/3 of the deprived cortex.

3.5.1 Resting membrane potential (V_m) was not affected by short sensory deprivation

The resting membrane potential of FS interneuron in control and deprived cortex was recorded a few minutes after breaking into the cell. The mean V_m was similar in control and deprived cortex and was not affected by deprivation (control: -64.0 [-61.5 – -69.5] mV, n = 23; deprived: -66.5 [-61.5 – -70.0] mV, n = 23; p = 0.516, Mann-Whithey Rank Sum Test) (Figure 3.3A).

3.5.2 Membrane resistance (R_m) was not affected by short period of sensory deprivation

The membrane resistance (R_m) of FS interneurons located in control (n = 26) and deprived cortex (n = 23) was calculated using Ohm's Law (see Section 2.4.3.4). The median R_m in

control cortex was 58 mΩ [48 - 73] MΩ and it was similar to the value reported by Beierlein et al. (Beierlein et al. 2003).

The R_m of FS interneurons in deprived cortex was 55 mΩ [45 - 68] MΩ and it was not significantly different from the control (p = 0.532; Mann-Whithey Rank Sum Test) (Figure 3.1B). Therefore, a short period of whisker trimming did not affect the R_m of FS interneurons in deprived cortex.

3.5.3 Membrane time constant (T_m) was not affected by short period of sensory deprivation

The membrane time constant (T_m) is defined as the time required to change the membrane potential by 1-(1/*e*) (~63 %) of the final voltage change and was extrapolated from the decaying exponential curve fitted to the change in membrane potential evoked by a - 0.1 nA current injection. The T_m of FS interneurons in deprived cortex was similar to the T_m of FS interneurons in control cortex (control cortex: 5.4 ± 0.3 ms, n = 26; deprived cortex: 5.0 ± 0.2 ms, n = 23; t= 1.055, p = 0.297, t-test) and it was not affected by the deprivation status (Figure 3.3C).

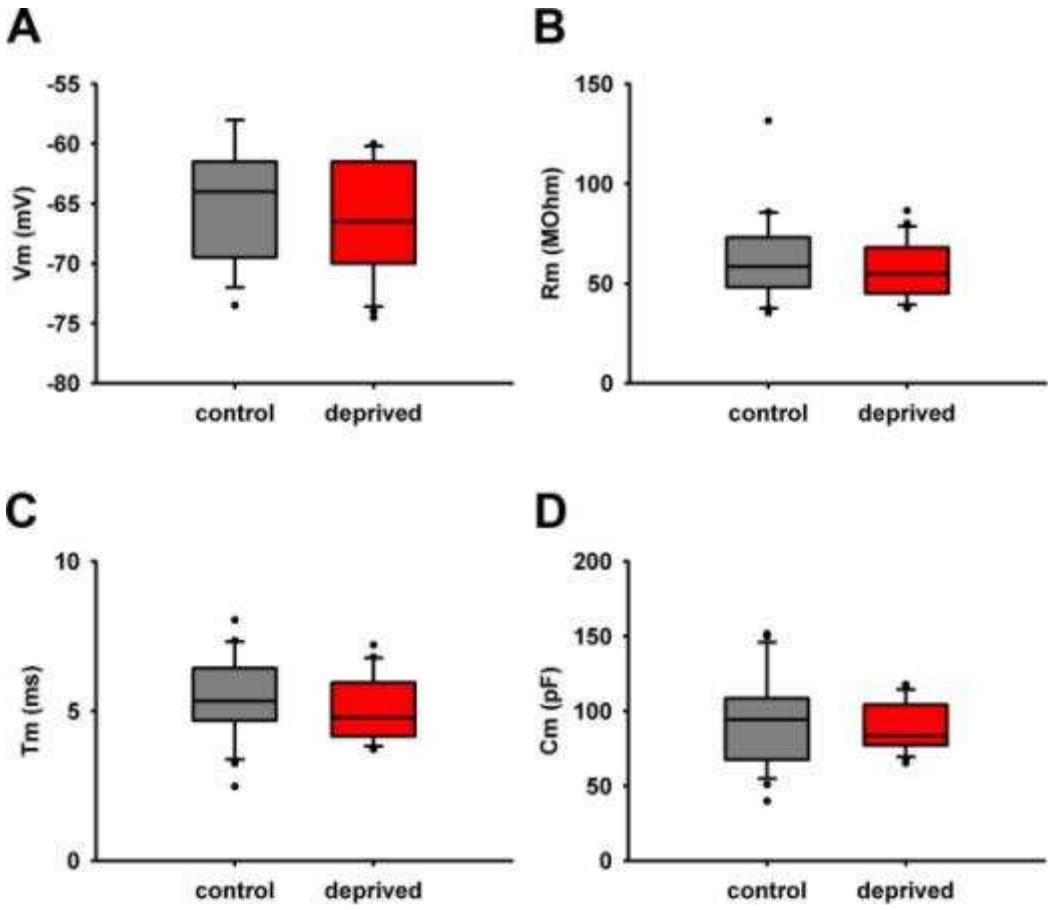

Figure 3.3 Passive membrane properties of layer 2/3 FS interneurons in control and deprived cortex.

***A**. Box and whisker plot of FS interneurons V_m in control (gray) and deprived (red) cortex after 2 - 3 days of whisker trimming. **B**. Box and whisker plot of FS interneurons R_m in control (gray) and deprived (red) cortex after 2 - 3 days of sensory deprivation. **C.** Box and whisker plot of T_m in FS interneuron from control (gray) and deprived (red) cortex after 2 - 3 days of sensory deprivation. **D.** Box and whisker plot of FS interneurons C_m in control (gray) and deprived (red) cortex after 2 - 3 days of whisker trimming.*

3.5.4 Membrane capacitance (C_m) was not affected by short period of sensory deprivation

The membrane capacitance of FS interneurons in control and deprived cortex was calculated using T_m and R_m. The C_m was not significantly different in deprived cortex

compared to control cortex after 2 - 3 days of whisker trimming (control cortex: 92 ± 6 pF, n = 26; deprived cortex: 90 ± 3 pF, n = 23; t = 0.245, p = 0.808, t-test) (Figure 3.3D).

3.6 Action potential shape

I compared the action potential shape in FS interneurons in deprived and control cortex by using the following parameters:

- Threshold: the membrane potential at which the rate of change dV/dt is greater than 20 V/s;
- Height: the difference in potential between the threshold and the AP peak;
- ISI: the time interval between two successive APs peaks;
- Latency: the time between the start of the current injection and the first AP peak;
- Half-width: the time distance between rising and descending phase at half of the AP height;
- AHP amplitude: the voltage difference between the threshold and the minimum membrane potential after the AP.

As some of these parameters were variable during the train, I averaged the measures of each parameter for the first and last 5 APs generated by a 500 ms square pulse of 1 nA current injection for each cell. The initial (average of the first 5 APs) and final (average of the last 5 APs) values for each parameter were calculated using a single trace for each cell (n = 26 control cells, n = 26 deprived cells). Initial and final values for each cell were therefore likely to be correlated; hence I performed statistical modelling using generalised estimating equations (GEE) to account for those correlations (see Section 2.6). The models increase the power of the statistics, as they prevent the use of multiple tests, which would require corrections. The models generated an expected value for the corresponding parameter measured, the expected changes associated with the deprivation status and the initial or final condition and the p-values derived from the robust z (see Section 2.6). The expected values for AP threshold, AP height, AP half-width and AHP amplitude and the

corresponding changes predicted by the models are reported in the following sections. Figure 3.4 and Figure 3.5 report instead whisker plots of the raw data for each initial and final parameter in both control and deprived cortex.

3.6.1 Threshold

The initial control AP threshold estimated by the model was -46.3 ± 1.1 mV µs (control: n = 26; deprived: n = 26). The final threshold was significantly depolarised (model parameter: γ = +4.90 ± 0.46 mV, $p < 0.001$) compared to the initial threshold. The deprivation status, in contrast, did not significantly affect the AP threshold (model parameter: β = -0.57 ± 1.53 mV, $p = 0.711$). The raw AP threshold data are plotted in Figure 3.4A.

These AP threshold values are more hyperpolarised compared with the - 36 mV threshold reported by Sun (Sun 2009). The discrepancy is probably attributable to the difference in recording temperatures. Although the threshold in my dataset changed during the train, a short period of sensory deprivation did not affect the AP threshold of layer 2/3 FS interneurons.

3.6.2 Action potential height

The estimated mean initial AP height of FS interneurons was 65 ± 1 mV in control cortex (n = 26) and 63 ± 1 in deprived cortex (n = 26). Deprivation status had no effect on AP amplitude (model parameters: β = -2.6 ± 1.6 mV, $p = 0.10$). AP amplitude significantly decreased with repetitive firing induced by current injection (+ 1.0 nA, 500 ms); the final AP height was 56 ± 1 mV in control and 53 ± 1 mV in deprived cortex (model parameter: γ = -9.8 ± 0.5 mV, $p < 0.001$). Figure 3.4B shows whisker plots of the raw initial and final AP amplitudes in control and deprived cortex.

The AP height measure I reported here are comparable to those reported by Povysheva et al (Povysheva et al. 2006) in rat prefrontal cortex but are smaller than those reported by Cauli et al. (Cauli et al. 1997) and Sun (Sun 2009) in somatosensory cortex. However, the studies I mentioned performed the recordings at room temperature; this would easily explain the differences in AP height measures.

3.6.3 Latency and interspike interval (ISI)

The action potential latency in control FS interneurons was not different from the latency in FS interneurons of deprived cortex (control latency: 1.5 [1.1 – 1.8] ms, n = 26; deprived latency: 1.5 [1.2 – 1.9] ms, n = 26; p = 0.956, Mann-Whithey Rank Sum Test). The initial ISI (control: 3.9 [3.2 – 4.1] ms, n = 26; deprived: 3.8 [3.0 – 4.2] ms, n = 26; p = 0.559, Mann-Whithey Rank Sum Test) and the final ISI (control: 3.8 [3.0 – 4.3] ms, n = 26; deprived: 3.3 [2.9 – 4.1] ms, n = 26; p = 0.498, Mann-Whithey Rank Sum Test) were similar in control and deprived cortex (Figure 3.4C). The ISI did not change during the train both in control (p = 0.940, Rank Sum Test) and in deprived cortex (p = 0.870, t-test). This is consistent with no frequency accommodation, typical of FS interneurons (Gupta et al. 2000, Markram et al. 2004, Kawaguchi 1995).

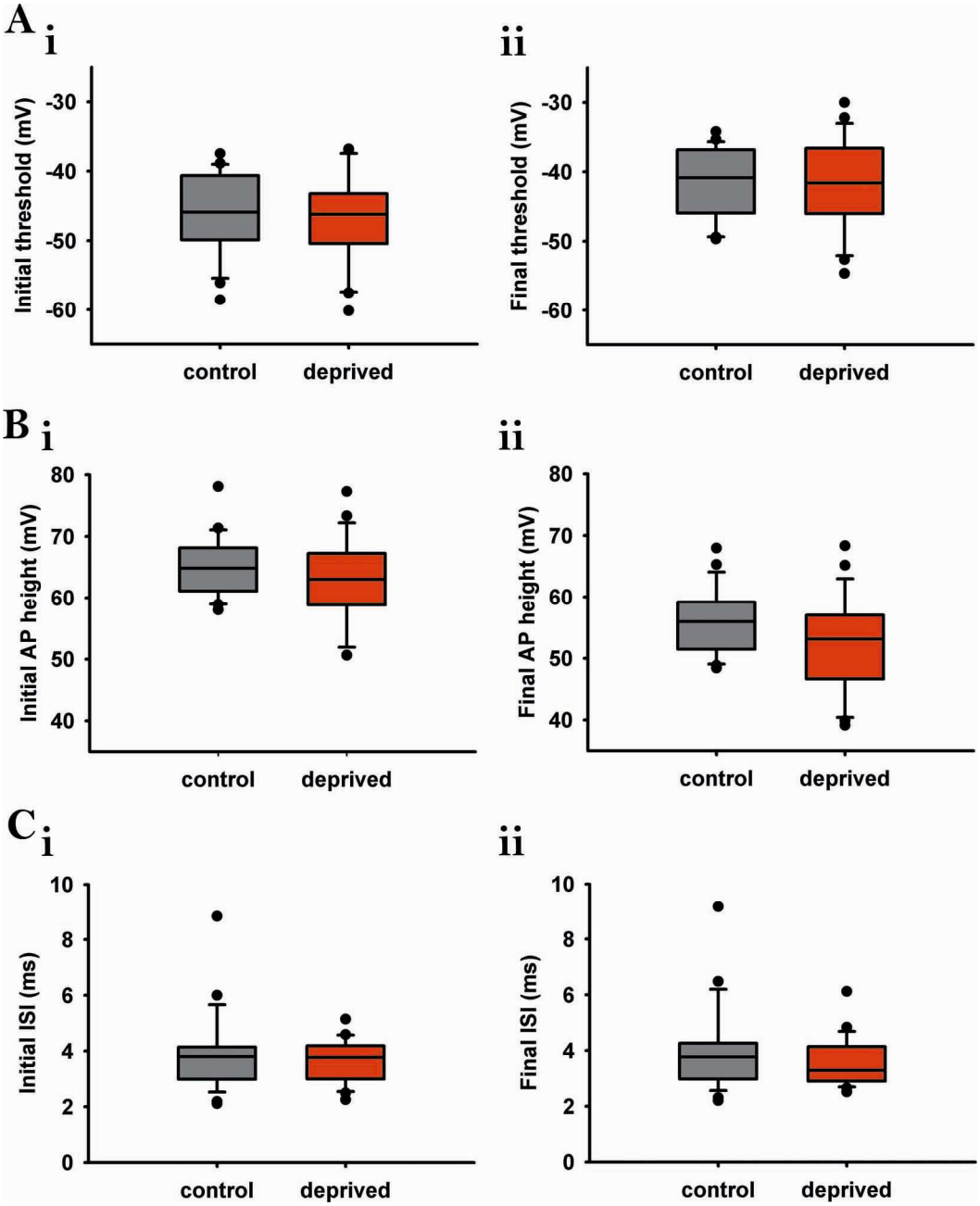

Figure 3.4 AP threshold, AP height and ISI interval were not affected by deprivation

***A**. Initial (i) and final (ii) AP thresholds in FS interneurons of control (gray) and deprived (red) cortex after 2 - 3 days of whisker trimming. Initial control threshold: -46.2 ± 1.1 mV (n = 26); final control threshold: -41.6 ± 0.9 mV; initial deprived threshold: -47.0 ± 1.2 mV (n = 26); final deprived threshold: -41.8 ± 1.3 mV. **B**. Initial (i) and final (ii) AP heights above threshold in FS interneurons of control (gray) and deprived (red) cortex. Mean control AP height: 65 ± 1 mV (n = 26) final control AP height: 56 ± 1 mV; mean initial deprived AP*

height: 63 ± 1 mV (n = 26); final deprived AP height: 52 ± 1 mV). ***C****. Initial (i) and final (ii) ISIs in FS interneurons of control (gray) and deprived (red) cortex after 2 - 3 days of whisker trimming.*

3.6.4 Action potential half-width

The initial estimated AP half-width in control cortex was 188 ± 5 µs (control: n = 26; deprived: n = 26). The AP half-width significantly increased in the final spikes compared to the initial ones (model parameter: γ = +32 ± 2 µs, p = 0.002). APs in FS interneuron of deprived cortex had a predicted initial AP half-width of 215 ± 5 µs. The deprivation status significantly increased the AP half-width (model parameters: β = +26 ± 3 µs, p < 0.0001).

The AP half-width values that I recorded were slightly shorter compared to the half-width reported for FS interneurons recorded at room temperature (Beierlein et al. 2003, Cauli et al. 1997, Kawaguchi and Kubota 1997, Povysheva et al. 2006, Avermann et al. 2012).

3.6.5 After hyperpolarisation phase (AHP)

The mean initial AHP amplitude in control cortex was 17.8 ± 0.7 mV µs (control: n = 26; deprived: n = 26). The AHP amplitude decreased during the train (model parameter: γ = -1.98 ± 0.68 mV, p = 0.004). The mean deprived AHP amplitude was also significantly reduced compared to control (model parameter: β = -3.20 ± 1.00 mV, p = 0.001). The raw AHP amplitude values are reported in Figure 3.5. I concluded that short period of sensory deprivation decreased the AHP of interneurons in deprived cortex.

The AHP amplitude in my control dataset was slightly smaller compared to the 24 mV reported by Cauli et al (Cauli et al. 1997) and the 27 mV reported by Povysheva (Povysheva et al. 2006). Those differences could, again, be related to different recording

temperature and different internal solutions (potassium gluconate versus potassium methylsulphate) used for the recordings.

3.6.5.1 The correlation of changes in AP half-width and AHP amplitude

A short period of sensory deprivation significantly increased the AP half-with and decreased the AHP amplitude in FS interneurons of layer 2/3. As both the parameters were measured from the same AP traces, I investigated whether the change in AHP amplitude was directly responsible for the change in half-with. When the half-width data were plotted versus the AHP amplitude data in control and deprived cortex, they showed a clear negative correlation (Pearson's correlation coefficient = -0.491; n = 104, $p < 0.0001$) (Figure 3.5C). Therefore, I modelled the estimated half-width including a continuous variable for the AHP amplitude and a continuous variable for the AP amplitude. The model showed that half-width increased as the AHP amplitude and the AP amplitude decreased (model parameter: $\alpha = 403 \pm 16$ µs, $p < 0.0001$; $\gamma = -1.01 \pm 0.33$ µs, $p = 0.003$; $\delta = -3.02 \pm 0.19$ µs, $p < 0.0001$). However, deprivation on its own also increased the half-width (model parameter: $\beta = 15.24 \pm 7.19$ µs, $p = 0.034$). Therefore, the change in half-width in deprived cortex was partially due to the decrease in AHP amplitude and AP amplitude, but it also occurred as a result of a short period of sensory deprivation.

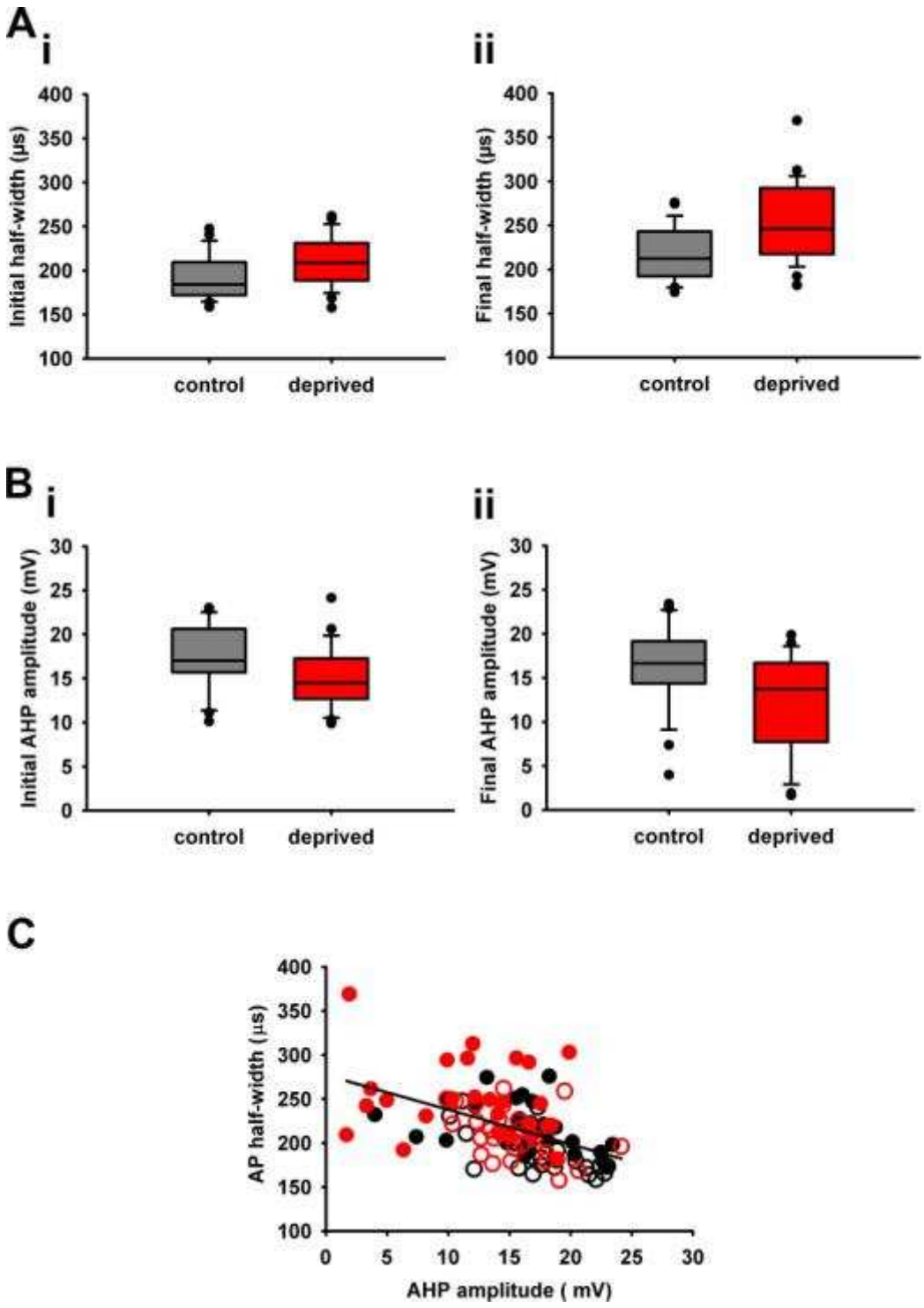

Figure 3.5 Short deprivation affected AP half-width and AHP amplitude

A. *Initial (i) and final (ii) AP half-widths in FS interneurons of control (gray) and deprived (red) cortex after 2 - 3 days of whisker trimming. Mean initial control half-width: 192 ± 5 µs,*

n = 26; mean initial deprived: 211 ± 6 µs, n = 26; final control AP half widths: 212 [192 – 243] µs, n = 26; final deprived: 246 [217 – 292] µs, n = 26. ***B****. Initial (i) and final (ii) AHP amplitudes in FS interneurons of control (gray) and deprived (red) cortex. Mean initial AHP amplitude control: 17.3 ± 0.7 mV, n = 26; initial deprived: 15.1 ± 0.7 mV, n = 26; final control AHP amplitude: 16.6 [14.4 – 19.2] mV, n = 26; final deprived: 13.7 [7.7 – 16.7] mV, n = 26.* ***C****. Scatter plot of AP half-width versus AHP amplitude in control (black; open circles = initial, filled circles = final) and deprived (red; open circles = initial, filled circles = final) cortex.*

3.7 Intrinsic excitability

The intrinsic excitability of layer 2/3 FS interneurons was measured by plotting the input-output curve, which shows how AP firing changes with current injections. The input-output plot of FS interneurons was linear in the range 0.4 – 1.0 nA (Figure 3.6B), therefore the slope and the intercept of the linear curve fitted to the data for each cell was used to compare control and deprived firing.

The average input-output functions for FS interneurons in control and deprived cortex are reported in Figure 3.6B. The mean input-output function slope in deprived cortex was not significantly different compared with the mean slope in control cortex (control: 186 ± 10 $AP.nA^{-1}$, n = 32; deprived: 182 ± 9 $AP.nA^{-1}$ n = 26; t = 0.291, p = 0.772, t-test) (Figure 3.6C). The mean intercept was also unaffected by short period of sensory deprivation (control: -35 ± 7, n = 32; deprived: -25 ± 8, n = 26; t = -0.971, p = 0.336, t-test) (Figure 3.6C).

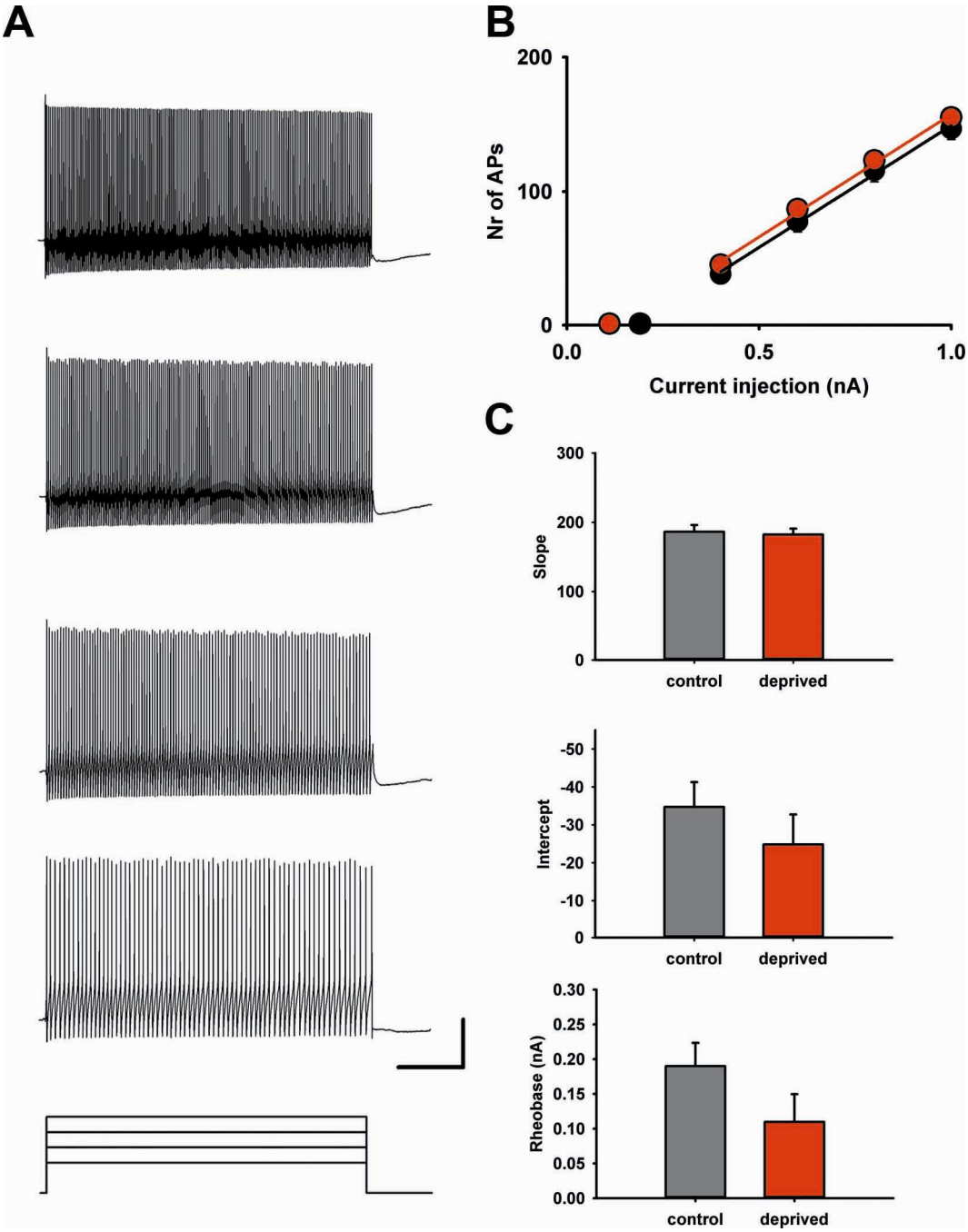

Figure 3.6 Intrinsic excitability of layer 2/3 FS interneurons in control and deprived cortex

***A**. Example traces of action potentials in response to 500 ms depolarising current pulses (0.4 – 1.0 nA) from a layer 2/3 FS interneuron. Scale bars: 100 ms, 20 mV**. B**. Input-output functions for layer 2/3 FS interneurons in control (black) and deprived (red) cortex after 2-3 days of whisker trimming. **C**. Bar charts of (from top to bottom): input-output function slope, intercept and extrapolated rheobase in control (gray) and deprived (red) cortex.*

After injecting currents in the range 0.4 – 1.0 nA, I injected smaller current to establish the rheobase, which is the current injection required to elicit one single AP. However, an accurate rheobase value was very difficult to obtain during the recordings, as the same current injection repeated twice, would elicit different numbers of APs. This could be due to variation in the resting membrane potential and baseline synaptic activity that was not suppressed while recording the excitability data.

Therefore, I decided to estimate the rheobase from the input-output curve, by imposing y = 1 in the input-output function. This estimates the current required to fire one action potential. The estimated mean rheobase in deprived cortex (0.110 ± 0.04 nA, n = 32) was not significantly different to the estimated mean rheobase in control cortex (0.190 ± 0.03 nA, n = 26; t = 1.556, p = 0.125, t-test) (Figure 3.6C).

3.8 Chapter summary

In this chapter I investigated the effect of 2 - 3 days of whisker trimming on the intrinsic properties of layer 2/3 FS interneurons in deprived and control cortex.

I found that:

- Both initial and final half-width were increased in deprived cortex compared with controls;
- Both initial and final AHP amplitude were decreased in deprived cortex after 2 - 3 days of whisker trimming;
- The passive membrane properties (V_m, R_m, T_m and C_m) of layer 2/3 FS interneurons were similar in contol and deprived cortex;
- The initial and final: threshold, AP height, ISI and latency were not affected by brief sensory deprivation;
- The slope and the intercept of the input output function, as well as the estimated rheobase, were not significantly different in deprived cortex compared with control.

3.9 Discussion

FS interneurons have distinctive passive membrane properties such as low membrane resistance and fast time constant compared to pyramidal cells. Those properties develop around the second or third postnatal weeks and they are mainly determined by the maturation of ionic leak conductances, in particular inwardly rectifying K^+ currents generated by Kir channels and some members of the TASK subfamily 2-pore K channels (Goldberg et al. 2011). In this chapter, I showed that passive membrane properties of FS interneurons were not affected by 2 - 3 days of whisker trimming in deprived cortex. Whisker trimming has been shown to decrease layer 4 FS interneurons R_m and increase T_m in deprived cortex, but those changes occurred only when trimming started at the end of the critical period (P7) and lasted for more than 2 weeks (Sun 2009). A short period of sensory deprivation in adulthood did not affect the passive membrane properties of layer 2/3 pyramidal cells neither in deprived nor in spared cortex (Barnes 2010). Longer period of deprivation (> 2 weeks) in adolescence also did not affect passive membrane properties of layer 2/3 pyramidal cells (Cheetham et al. 2007) and FS interneurons (Edwards 2008). Taken together, these results suggest that adult cortical plasticity in layer 2/3 is unlikely to be mediated by changes in the passive membrane properties of neocortical cells.

FS interneurons of layer 2/3 primary somatosensory cortex have peculiar electrophysiological firing properties that comprise: very high frequency, no frequency adaptation, no or little spike accommodation and large, fast AHP (McCormick et al. 1985, Kawaguchi 1995). This mature firing behaviour of neocortical layer 2/3 FS interneurons also develops between the second and the third postnatal week but it is determined by the expression of a specific subset of potassium channels belonging to the Kv3 subfamily (Goldberg et al. 2011, Rudy and McBain 2001). Kv3.1 and Kv3.2 are channels highly expressed in parvalbumin-positive FS interneurons (Chow et al. 1999); they are activated only at very depolarized membrane potentials (above -10 mV) (Rudy 1999) and they have very fast activation and deactivation rates, which makes them crucial in sustaining the high firing frequency of this cell type. Pharmacological experiments have shown that Kv3.1 and

Kv3.2 blockage reduces the AHP amplitude and increases the AP half-width (Erisir et al. 1999). The pharmacological inactivation of Kv3.1 and Kv3.2 also decreases the steady state firing frequency of FS interneurons but does not affect the initial firing frequency, suggesting that these channels play a vital role in limiting the inactivation of Na^+ channels (Erisir et al. 1999).

2 - 3 days of whisker trimming did change some of the AP shape parameters: both the initial and final AHP amplitude decreased in deprived cortex and both the initial and final AP half-width increased as a result of sensory deprivation. Those changes were consistent throughout the train and became more prominent in the final APs. As the half-width was increased in deprived cortex, this could possibly be responsible for the decrease in AHP amplitude in deprived cortex, as the two measures were inversely correlated. Therefore, I generated a statistical model for the AHP amplitude that included AP half-width. The model showed that increased half-width predicted a decrease in AHP amplitude. However, it did not account for the whole change. In fact, the deprivation status also affected the AHP amplitude suggesting that deprivation affected both half-width and AHP amplitude. The combination of these changes in AHP amplitude and half-width could then be consistent with a reduction in the density of Kv3.1 and/or Kv3.2 potassium channels. To support this hypothesis, similar changes in AP shape can be obtained by washing low concentration (0.1 mM) of TEA, which blocks a fraction of K+ channels, including Kv3 channels (Erisir et al. 1999). In my dataset, there was no change in the initial or in the final steady state ISI in deprived cortex. However, it could be possible that the change in channel density at this time point is not great enough to compromise the recovery of Na^+ channel from the inactive state.

The changes in AP half-width and AHP amplitude would predict a change in repetitive firing. However, brief period of sensory deprivation did not change the initial or the final firing threshold of FS interneurons in deprived cortex and did not affect the initial and final inter spike interval. The neuronal excitability was also unaffected, as there were no

changes in the slope and the intercept of the input-output function. The mean input-output function in deprived cortex was slightly shifted towards bigger action potential numbers, but the shift was not significant. Moreover, the baseline synaptic activity was not blocked during the recordings; therefore, changes in synaptic activity (see Chapter 4) could have interfered with the measure of the intrinsic excitability.

Changes in AP shape and spike broadening can affect neurotransmitter release at the synapses (Wheeler et al. 1996). This is extremely relevant considering that Kv3.1 and Kv3.2 channels are expressed not only in the soma but also in FS interneurons synaptic terminals (Chow et al. 1999). In fact, application of saturating concentration of TEA to rat brain slices has been shown to increase the amplitude of uIPSPs recorded from pairs of synaptically connected FS interneurons - pyramidal cells of layer 2/3 barrel cortex and decrease the pair pulse ratio (PPR) (Goldberg et al. 2005). Therefore, the small but still significant changes in APs shape that were induced by brief period of sensory deprivation could be important and affect neurotransmitter release and short term synaptic dynamic of FS interneuron connections in layer 2/3 of barrel cortex. This topic will be further investigated in Chapter 5.

Chapter 4 Experience-dependent plasticity of miniature postsynaptic events

4.1 Introduction

Previous work in my lab showed that a short period of whisker trimming affects the excitatory circuit in layer 2/3 deprived cortex (Barnes 2010). Specifically, whisker trimming increases the connectivity between pyramidal cells of layer 2/3 deprived cortex. As mature circuits require a fine balance between excitation and inhibition, here I explored whether inhibitory circuits were also modified by short sensory deprivation.

The reorganization of circuitscould involve either changes in the intrinsic properties of cells or changes in the excitatory/inhibitory inputs received by each cell. A short period of deprivation did not affect the intrinsic properties of FS interneurons in layer 2/3 (see Chapter 3). Therefore I investigated whether short sensory deprivation affected the global excitatory drive onto FS interneurons. I did this by recording miniature excitatory postsynaptic potentials (mEPSPs) from layer 2/3 FS interneurons in control and deprived cortex. Deprivation could also alter the inhibitory circuits by changing the overall inhibitory drive onto excitatory cells. Hence, I also investigated whether deprivation affected the global inhibitory drive onto layer 2/3 pyramidal cells. The overall inhibitory drive onto pyramidal cells was assessed by recording miniature inhibitory postsynaptic currents (mIPSCs) from layer 2/3 pyramidal cells in control and deprived cortex.

4.2 Background

Spontaneous and miniature postsynaptic events have been previously used to assess changes induced by experience-dependent plasticity. However, not much is known about the effects of deprivation on the inhibitory circuitry in adult animals. When studying plasticity it is important to distinguish between developmental and mature circuits, as the plasticity mechanisms involved during development are likely to be different from the ones involved in the reorganisation of mature circuits (Albieri and Finnerty 2012, Barnes and Finnerty 2010).

Whisker trimming is a very good paradigm to induce deprivation, as it is innocuous and does not induce deafferentation (Li et al. 1995). Whisker trimming significantly affects inhibitory circuits in layer 4 of primary somatosensory cortex during development (Jiao et al. 2006). In fact, prolonged whisker trimming (> 2 weeks) of a single row of whiskers starting at P7 induces a decrease of parvalbumin expression in interneurons of deprived cortex (Jiao et al. 2006). Parvalbumin-positive cells also show a decrease in the number of boutons, which is supported by a reduction in amplitude and frequency of mIPSCs recorded in spiny neurons (Jiao et al. 2006). The same protocol also decreases the frequency of sEPSCs in FS interneurons of layer 4, with no changes in amplitude. However these changes in layer 4 are strictly developmental, as no change in parvalbumin-expression was found when trimming started at P15 (Jiao et al. 2006).

In visual cortex, 2 days of monocular deprivation by intraocular TTX injection starting at P14 results in an increase in mEPSCs amplitude in layer 4 excitatory cells of the contralateral monocular cortex (Desai et al. 2002). A similar effect can also be induced in cultured neurons, where pharmacological manipulations of activity levels results in compensatory changes in the amplitude of excitatory synapses (Turrigiano 1999). In both cases the changes are multiplicative, and the whole distribution of miniature events is shifted. This type of change has been referred as synaptic scaling. Synaptic scaling is a homeostatic mechanism of plasticity, as it acts to oppose a global change in activity levels. Homeostatic synaptic scaling also preserves the relative difference in strength between synaptic contacts, as all the events are scaled up or down by a constant factor. While Hebbian plasticity requires a correlation between pre- and post- synaptic activity that results in strengthening of specific connections, synaptic scaling affects each synaptic contact equally and therefore has been described as non-Hebbian mechanism of plasticity (Turrigiano 2008).

Monocular deprivation starting at P14 not only increases the excitatory drive on star pyramidal cells of layer 4, it also decreases the synaptic inhibitory drive (Maffei et al.

2004). FS interneurons are particularly affected by the deprivation, as monosynaptic connections between FS interneurons and star pyramid cells in deprived cortex show a decrease in evoked IPSC amplitude and changes in short term synaptic dynamics. However, experience-dependent changes to the inhibitory circuitry induced by monocular deprivation during the critical period in layer 4 are substantially different to the changes reported in layer 4 during the pre-critical period (Maffei et al. 2004, Maffei et al. 2006). In fact, 2 days of monocular deprivation starting at P18 induces strengthening of the feedback circuit involving FS interneurons and pyramidal cells (Maffei et al. 2006).

Monocular deprivation during the critical period (P18 – P21) also affects circuits of layer 2/3 (Maffei and Turrigiano 2008). However, the nature of the changes in layer 2/3 depends on the type of deprivation protocol applied (Maffei and Turrigiano 2008). TTX injection, in fact, increases the total spontaneous excitatory drive on pyramidal cells and decreases the overall inhibitory drive (Maffei and Turrigiano 2008). In contrast, lid suture at the same time-point decreases the excitatory drive and leaves the inhibitory drive unaltered (Maffei and Turrigiano 2008). Unlike changes in layer 4, changes in layer 2/3 persist in adulthood, as dark rearing for two days induces mEPSC amplitude increase in layer 2/3 pyramidal cells when deprivation is started at P36 and P95 (Goel and Lee 2007). However, in adult mice the multiplicative scaling effect is lost, suggesting that the mechanisms behind those changes could be different to those occurring in young animals (Goel and Lee 2007).

A few recent studies have also investigated the effect of experience-dependent plasticity in visual cortex of adult animals. A permanent focal retinal lesion in adult mice generates a temporary loss of responsiveness in the corresponding portion of monocular cortex, which reorganizes structurally and functionally (Keck et al. 2008, Keck et al. 2011). The dynamics of spines and boutons of spiny interneuron in layer 2/3 are altered by this deprivation protocol, with a net decrease of both spines and boutons. Complete retinal lesion also induced functional changes in the deprived area (Keck et al. 2011). In fact, mIPSCs recorded from pyramidal cells in the corresponding layer 5 monocular visual

cortex after complete lesion show a decrease in frequency, compatible with the decrease in boutons. However, the structural changes induced by focal retinal lesion occur on a different timescale compared to those generated by complete lesion (Keck et al. 2011).

Structural loss of inhibitory synapses in layer 2/3 of adult mice following 5 days of monocular deprivation has also been inferred by in vivo imaging of GFP-gephyrin puncta onto layer 2/3 pyramidal cells of adult mice during 12 days of monocular deprivation, although functional changes were not investigated (van Versendaal et al. 2012).

In barrel cortex a long period of whisker trimming starting at P19, just after the end of the critical period of development for the layer 2/3 map, induces deep changes on excitatory circuits (Cheetham et al. 2007). Specifically, excitatory neurons located in deprived cortex, at the border with the spared column, exhibit increased mEPSPs amplitude but also dramatic decrease in local excitatory connectivity. After two days of deprivation however, the excitatory cells in deprived cortex undergo a transient threefold increase in local excitatory connectivity (Barnes 2010). A similar deprivation protocol induces expansion of the BOLD - fMRI signal following deflexion of the spared whiskers (Alonso et al. 2008). This evidence suggests that after two days of deprivation the circuits in deprived cortex initiate a process of reorganization. The increased connectivity within excitatory circuits, by generating positive feedback loops, is likely to shift the balance between excitation and inhibition, unless inhibitory circuits are also altered by deprivation. Hence, here I investigated whether the inhibitory circuits adapt to follow the excitatory changes or the alteration of the balance between excitation and inhibition is a requirement of the reorganization process.

4.3 Methods summary

Sprangue Dawley rats were deprived of sensory input by trimming a subset of whisker rows. Trimming started at P30 and was performed daily for 2 or 3 days. Control animals were sham-trimmed daily. Animals were anesthetized and perfused with ice-cold dissection ACSF. Slices were kept in bACSF for 1hour at 36˚C and then let to cool down to room temperature. Recordings were performed at 37˚C under constant flow of bath ACSF. To record mEPSPs, FS interneurons were patched with a K-based internal solution (see Section 2.3). 1 µM Tetrodotoxin (TTX) and 100 µM picrotoxin (PTX) were applied to the slice to abolish action potential firing and block $GABA_A$ receptor mediated potentials respectively. Traces of 5 s each were acquired using an 8-pole Bessel filter with a -3dB corner frequency of 3 kHz. mEPSPs analysis was performed using custom written Labview program. 50 events were analyzed for each cell. mIPSCs were recorded from pyramidal cells in voltage clamp mode using a Cs-based internal solution containing 10 mM lidocaine bromide (QX314) to block leakage currents and increase the membrane resistance of the recorded neurons. The membrane potential of the neuron was clamped at 0 mV. Therefore mIPSCs were recorded as positive currents. 1 µM TTX, 50 µM APV and 20 µM CNQX were applied to the slice to abolish action potential firing and to block glutamate receptors. Traces were acquired in 10 seconds epochs using an 8-pole Bessel filter with a -3dB corner frequency of 2 kHz. mIPSCs were analyzed using a custom written program in Labview by examiner blind to the trim condition.

4.4 Miniature postsynaptic events

Miniature postsynaptic events were first discovered by Fatt and Katz at the neuromuscular junction of the frog (Fatt and Katz 1952). The spontaneous sub threshold variations of the membrane potential recorded from the postsynaptic terminal of an active chemical synapse occur: (i) in response to spontaneous action potentials: (ii) and because of activity-independent stochastic release of vesicles of neurotransmitters from the presynaptic terminal (Connors et al. 1982). The probability of release for a vesicle in absence of activity is very low, but greater than zero. The desynchronized stochastic

release of vesicles can be recorded in form of miniature postsynaptic events. Miniature events can be isolated by blocking action potential firing in the slice through the administration of Tetrodotoxin (TTX), a reversible sodium channel blocker. The frequency of miniature postsynaptic depolarisations (or hyperpolarisations, if the neurotransmitter released is inhibitory) and the amplitude of those events can be used as indicative measure of the strength of the overall excitatory (or inhibitory) drive onto a cell. Miniature postsynaptic events recorded at the soma reflect global inputs and have certainly the limitation of being unspecific, i.e. it is not possible to discriminate which layers the inputs come from. Moreover, their interpretation is often difficult. For these reasons, miniature postsynaptic events are used in this thesis as a first approach to screen for changes in cortical circuits.

4.5 Miniature recordings in layer 2/3 fast-spiking interneurons

Miniature excitatory postsynaptic potentials (mEPSPs) were recorded from FS interneurons in layer 2/3 of deprived and control cortex. I recorded miniature events in current clamp mode. mEPSPs were recorded from animals aged P32 - P33 for both control and deprived animals. FS interneurons were selected only on the basis of morphological properties, hence the electrophysiological characterization of their firing pattern was indispensable to unequivocally identify them.

Because of the slow kinetics of the PSPs compared with the decay time of PSCs, the recordings in voltage clamp mode are more likely to recover more events than in current clamp (Simkus and Stricker 2002). However, voltage clamp recording requires a caesium based internal solution containing QX314 in order to overcome space clamp limitations. This internal solution affects the firing pattern of the cells even when the pipette is tip-filled with K^+ based solution, making cell identification impossible. Therefore I opted for recording mEPSPs.

4.5.1 Isolation of miniature excitatory postsynaptic potentials (mEPSPs)

Spontaneous sub threshold depolarisations of the membrane potential are the combined effect of action potential dependent and independent vesicle release. In order to isolate the stochastic quantal release, I recorded events after the application of the reversible sodium channel blocker TTX to block action potential firing in the slice. I checked this by applying 500 ms current pulses of 0.8 nA every 2 minutes after the application of TTX in the bath.

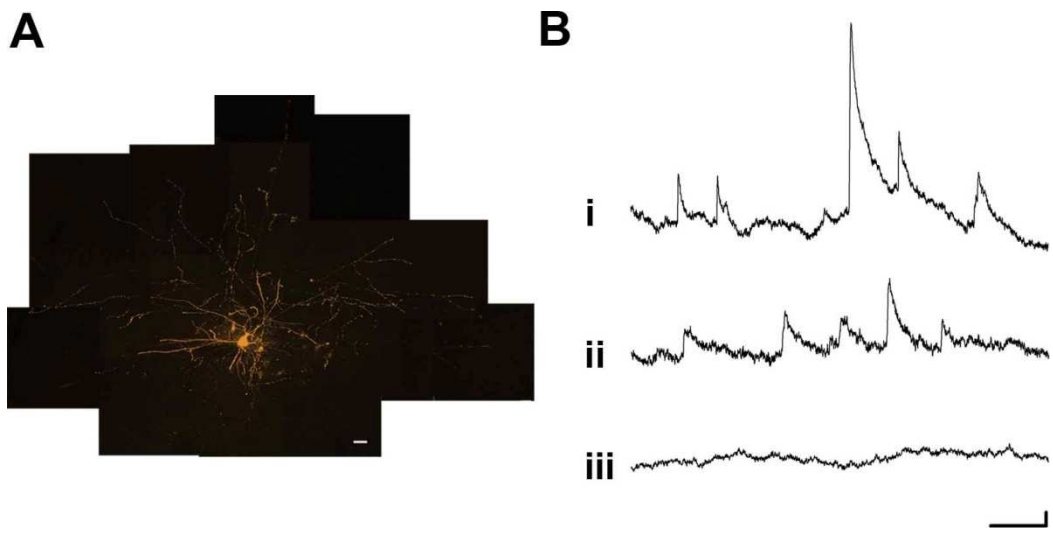

Figure 4.1 mEPSPs recordings in FS interneurons.

***A**. Montage of FS interneuron filled with AF568 fluorescent dye obtained by assembling maximum intensity projections from confocal stacks. Scale bar: 20 µm. **B**. Examples of: (i) spontaneous activity recorded from FS interneuron in deprived cortex, (ii) excitatory mEPSPs recorded 15 minutes after the bath application of 1 µM TTX and 100 µM PTX, (iii) mPSPs were completely abolished after the application of 1 µM TTX, 100 µM PTX, 20 µM CNQX and 50 µM APV. Scale bars: 0.2 mV; 20 ms.*

Action potential firing of the recorded cells was blocked approximately 7 minutes after drug application. PTX was also added into the bath to block GABA-A receptor mediated chloride currents, although their reversing potential is very close to the resting membrane potential of the cells. In order to develop the mEPSP acquisition protocol, I established the kinetics of drugs action and the stability of recordings over prolonged period of drug application. I analyzed mEPSP frequency and amplitude at regular intervals in presence of TTX and PTX (Figure 4.1). Both frequency and amplitude were stable from 5 to 30 minutes after the drugs application. The action kinetics of PTX was also tested, by analyzing the frequency and amplitude of mIPSCs at regular intervals after the application of TTX, CNQX and APV (Figure 4.7). The events were completely abolished 8 minutes after the application of PTX. mEPSPs were acquired 15 minutes after the bath application of TTX and PTX.

4.5.2 Short deprivation increases mEPSPs amplitude in FS interneurons of deprived cortex

Two days of whisker trimming significantly increased the mean amplitude of mEPSPs in FS interneurons of layer 2/3 deprived cortex (0.37 ± 0.01 mV, n = 9, p = 0.02, t = -2.54; t-test) compared to aged matched control (0.32 ± 0.02 mV, n = 13) (Figure 4.2A). The distribution of mEPSP amplitude was also shifted towards bigger amplitudes ($P < 0.001$, Mann-Whitney Rank Sum Test) (Figure 4.2B). Those changes could not be attributed to changes in passive membrane properties as there was no difference in membrane potential or input resistance between control and deprived cells (see Section 4.5.7, Table 4.1).

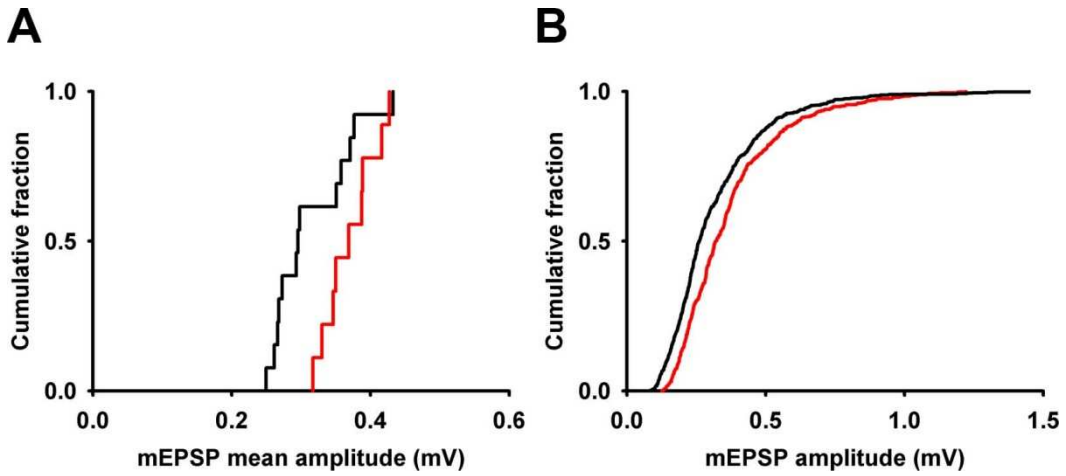

Figure 4.2 mEPSPs amplitude is increased in deprived cortex after brief sensory deprivation.

***A**. Cumulative fraction plot of mean mEPSPs in deprived (red, n = 9 neurons) and control (black, n = 13 neurons) cortex after 2 - 3 days of whisker trimming. **B**. Empirical distribution of all mEPSPs events in deprived (red, n = 450 events) and control (black, n = 650 events) cortex after 2 - 3 days of whisker trimming.*

4.5.3 mEPSPs frequency in deprived cortex is not altered by 2 - 3 days of deprivation

I recorded mEPSPs in FS interneurons located in layer 2/3 of control and deprived cortex. Two days of deprivation did not affect the frequency of mEPSPs in deprived cortex (15.2 [6.19 – 20.1] Hz, n = 9, p = 0.894; Mann-Whitney Rank Sum Test) compared to control cortex (12.7 [8.79 - 18.3] Hz, n = 13) (Figure 4.3).

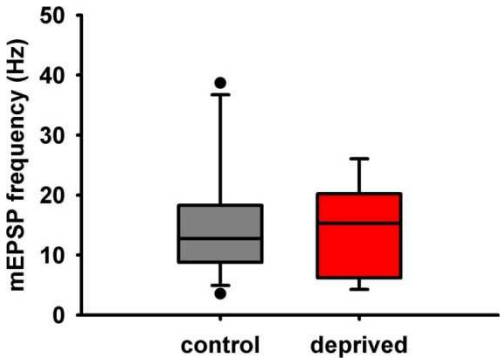

Figure 4.3 mEPSPs frequency is not altered by 2 - 3 days of sensory deprivation.

Box and whisker plot of mEPSP frequency in control (dark gray, n = 13 neurons) and deprived (red, n = 9 neurons) cortex after 2-3 days of sensory deprivation. Filled circles are values outside the 10th – 90th percentile range.

4.5.4 mEPSPs amplitude in deprived cortex does not increase multiplicatively

2 - 3 days of whisker trimming increased the average mEPSP amplitude in deprived cortex. Those changes could be either the result of anti-Hebbian synaptic plasticity at specific synapses or the global up-scaling of all synaptic contacts, a phenomenon described as homeostatic plasticity. Homeostatic up-scaling of glutamatergic connections has been previously reported after activity blockade in cultures (Turrigiano et al. 1998) and after monocular deprivation in slices (Desai et al. 2002). After 2 days of monocular deprivation started at P36, the distribution of mEPSCs amplitude was shifted towards bigger values by a single multiplicative factor (Goel and Lee 2007). In order to test whether the changes I found in deprived cortex could be of homeostatic nature, I plotted in ascending order 50 mEPSPs events for nine deprived cells against the same data for nine randomly selected control neurons (Figure 4.4). The optimal fit for the control versus deprived mEPSPs amplitude was a linear equation with intercept = 0.06 and slope = 1.00. This suggests that mEPSPs amplitude in deprived cortex does not increase in a multiplicative mode, but the shift is instead additive. I concluded that the change in mEPSP amplitude in SI were different from these reported in primary visual cortex (VI) following monocular deprivation.

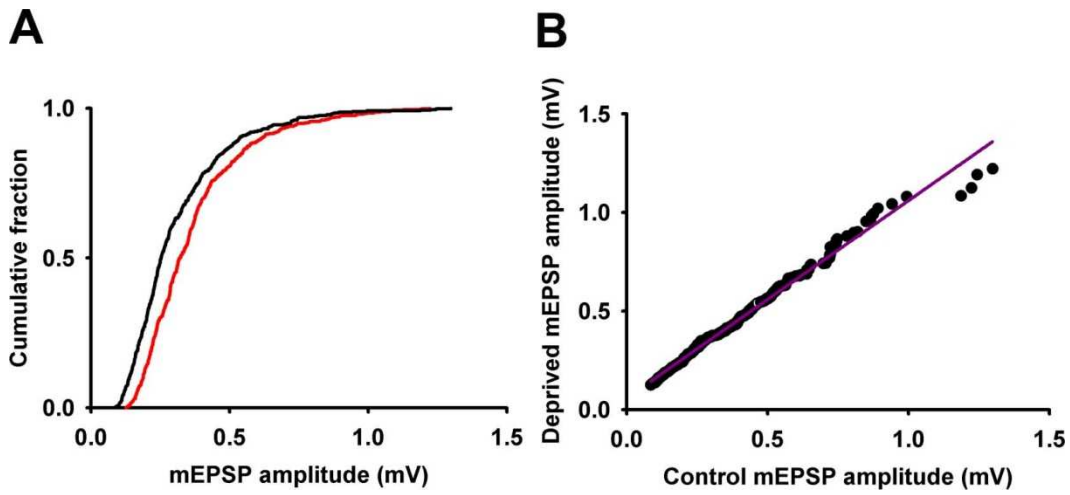

Figure 4.4 mEPSPs amplitude in deprived cortex is not scaled up multiplicatively

***A**. Cumulative fraction plot of all mEPSP events from nine FS interneurons in control cortex (black) and nine deprived cells (red). **B**. Scatter plot of ordered mEPSPs events amplitude in control cortex (450 events from nine randomly chosen cells, as in A) versus ordered mEPSPs event amplitude in deprived cortex (450 events from nine recorded cells, as in A.). The distribution is well fitted by the linear regression given by the equation* $y = 1.001x + 0.058$

4.5.5 Relationship between amplitude and frequency of mEPSPs in control and deprived cortex

During the critical period of development, mEPSC amplitude and frequency both in layer 4 and in layer 2/3 commonly change in opposite direction, with frequency increasing and amplitude decreasing (Desai et al. 2002). Therefore, I tested the relationship between amplitude and frequency in my dataset (Figure 4.5). In control cortex there was no correlation between mEPSP amplitude and frequency ($r(13) = -0.19$, $p = 0.526$). However, in deprived cortex, I found a negative correlation between amplitude and frequency ($r(9) = -0.82$, $P = 0.006$). As negative correlation has been previously related to homeostatic scaling in visual cortex, the possibility that the increased mEPSPs amplitude is due to

homeostatic up-scaling cannot be ruled out, although no multiplicative effect was found in my dataset. The existence of this negative correlation is however not sufficient to confirm the presence of homeostatic plasticity.

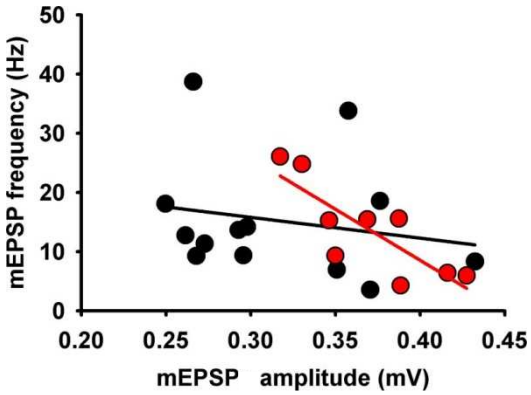

Figure 4.5 mEPSPs amplitude and frequency are negatively correlated in deprived cortex

Scatter plot of mean mEPSPs amplitude versus mEPSPs frequency in deprived (red, n = 9 neurons) and control cortex (black, n = 13 neurons) after 2 - 3 days of whisker trimming. Red and black lines depict linear regression lines for deprived and control groups respectively.

4.5.6 The mEPSPs amplitude is not related to the position of the neuron in the deprived column

Layer 2/3 neurons have different properties and receive different input according to their location within the layer (Bureau et al. 2006). Superficial neurons receive input mainly from layer 5 and layer 2/3 whereas deeper layer 2/3 cells predominately receive input from layer 4 (Bureau et al. 2006). Moreover, the degree of plasticity in a cell could be affected by its distance from the spared – deprived border (Cheetham et al. 2007). Therefore I investigated whether control and deprived cells were recorded in similar location and whether there was a relationship between location and mEPSP amplitude. The distance of

each interneuron from the pia and from the border between spared and deprived cortex are reported in Figure 4.6A. The location of the cells was similar in both control and deprived cortex (distance from border: p = 0.122, t-test). No relationship was found between mEPSP amplitude and the distance from the border in control (r(11) = 0.16, p = 0.637) or in deprived cortex (r(9) = -0.04, p = 0.921) (Figure 4.6B). This would suggest that the changes in inhibition, unlike the increase in mEPSPs of excitatory cells in deprived cortex after longer period of trimming (Cheetham 2007), occur uniformly within the deprived barrel closer to the border. No data were acquired from other deprived barrels; therefore the extent of the changes induced by whisker trimming outside the first deprived column is unknown.

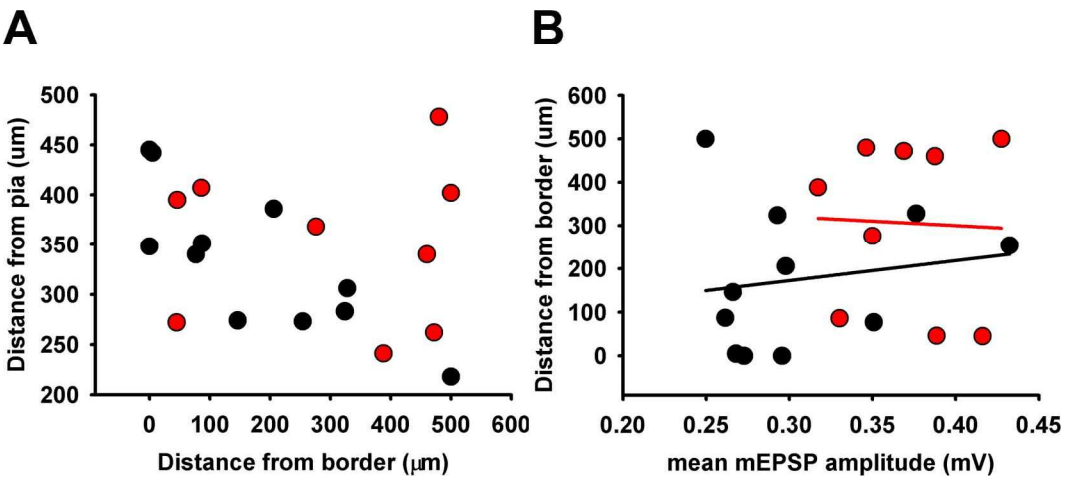

Figure 4.6 Location of the neurons in the barrel columns

A. *Scatter plot of the distance of the neuron from the pia against the distance from the border between spared and deprived cortex. Red dots represent cells in deprived cortex, black dots are control neurons.* ***B****. Scatter plot of mean mEPSPs amplitude versus distance from the border between spared and deprived cortex for deprived (red) and control (black) cells. Linear regression lines are reported following the dots colour scheme.*

4.5.7 Passive membrane properties

The amplitude and frequency of EPSPs are affected by the passive membrane properties. Therefore I measured the membrane resistance, resting membrane potential, capacitance and time constant of the cells used in the mEPSPs analysis are reported in Table 4.1. No significant difference between control and deprived groups was found in any of the properties reported.

Table 4.1 Passive membrane properties were not affected by short deprivation

	V_m (mV)	**R_m (MΩ)**	**T_m (ms)**	**C_m (pF)**
Control	-65 ± 1.3	58 (55 – 84)	6 ± 0.3	94 ± 6.8
Deprived	-67 ± 1.7	65 (59 – 74)	5.6 ±0.4	87 ± 4.4
Statistics	P = 0.417 (t – test)	P = 0.690 (Mann-Whitney)	P = 0.393 (t – test)	P = 0.458 (t – test)

Passive membrane properties of layer 2/3 FS interneurons in control and deprived cortex. Membrane potential (V_m), membrane resistance (R_m), time constant (T_m) and membrane capacitance (C_m) were not significantly different between the two groups.

4.6 mIPSC recording in layer 2/3 pyramidal cells

mIPSCs were recorded from pyramidal cells in layer 2/3 in control cortex and in deprived cortex after 2 - 3 days of deprivation. I chose to record inhibitory postsynaptic events in voltage clamp mode because at membrane resting potential inhibitory events are very hard to detect. This is because $GABA_A$ receptors are ionotropic channels permeable to small anions (mainly Cl^-), whose reversing potential in mature neocortical neurons is very close to the resting membrane potential of the cells. Therefore, in order to isolate inhibitory events, it is necessary to shift the membrane voltage to more negative or more positive values. Although it would still be possible to hyperpolarize and depolarize the membrane potential of the cell in current clamp, an earlier pilot study showed difficulties in isolating events from noise. Therefore, in this thesis I recorded inhibitory postsynaptic events in voltage clamp mode, holding the pyramidal neurons voltage at 0 mV. As a result, inhibitory postsynaptic events were recorded as outward currents Figure 4.7.

4.6.1 Isolation of miniature inhibitory postsynaptic currents (mIPSCs)

Miniature inhibitory postsynaptic currents (mIPSCs) were isolated by applying the sodium channel blocker TTX. In the presence of 1 µM TTX, the amplitude of the postsynaptic currents was notably reduced compared with spontaneous depolarisations. This is due to the loss of spontaneous action potential mediated postsynaptic currents. The recorded currents were then considered miniature inhibitory events (Figure 4.7). mIPSC recordings were also performed in the presence of the AMPA and NMDA receptors antagonists CNQX and APV, although the effect of excitatory transmission at 0 mV is minor. Bath application of the $GABA_A$ receptor antagonist PTX completely abolished mIPSCs (Figure 4.7B). Based on the kinetics of drugs action previously investigated for mEPSPs, data were collected at least 15 minutes after the bath application of the drugs. mIPSCs were selected visually, based on the criteria previously described, by an examiner blinded to the trim condition of the animals (see Section 2.4.4.3).

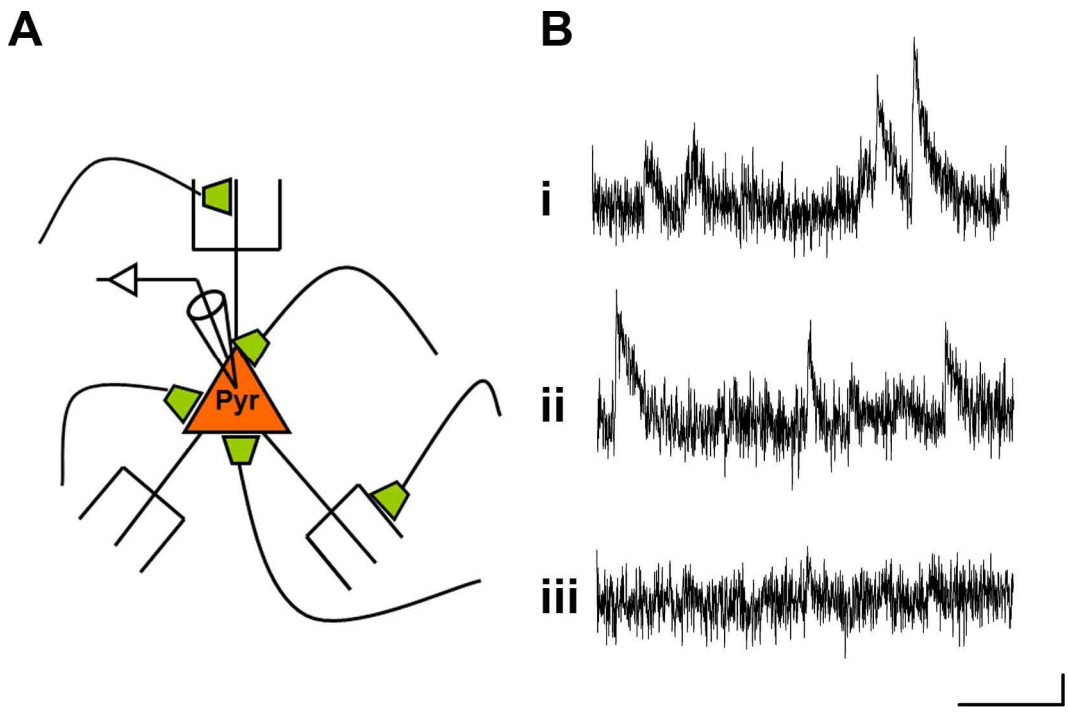

Figure 4.7 mIPSC recordings in layer 2/3 pyramidal cells

A. *Schematic of mIPSC recording. A layer 2/3 pyramidal cell (orange) is recorded in whole-cell voltage clamp mode. By clamping the membrane potential at 0 mV in the presence of TTX, CNQX and APV, inhibitory synaptic inputs can be recorded (as shown in B). Inhibitory synaptic inputs are schematised in green. Interneuron bodies are not included, to emphasise the limitations of voltage clamp recordings in distinguishing interneuron subtypes and the layer from which inputs originate.* ***B.*** *Examples of: (i) mIPSCs recorded from a layer 2/3 pyramidal cell in control cortex in presence of TTX, CNQX and APV; (ii) mIPSCs recorded from a layer 2/3 pyramidal cell in deprived cortex; (iii) no currents recorded after the application of the* $GABA_A$ *receptor antagonist PTX. Scale bars: 5 pA, 50 ms.*

4.6.2 Statement of contribution

The work in this chapter is all my own except for the mIPSC data from 5 control cells that were recorded by Dr. Clarissa Edwards. The 5 cells were pooled together with 10 cells I recorded to form the mIPSC control group used in this chapter.

4.6.3 mIPSCs amplitude is unaltered by short sensory deprivation

mIPSPCs were recorded from 16 cells in deprived cortex (8 animals) and 15 cells in control cortex (9 animals). All the data were collected from animals aged P32 - P33. Whiskers trimming (in deprived animals) started at P30 and lasted for 2 or 3 days.

The mean mIPSCs amplitude was 26.3 ± 1.1 pA in control cortex and 28.1 ± 1.4 pA in deprived cortex. The mean amplitude was not significantly different between the two groups ($p = 0.330$, $t = 0.991$, t- test) (Figure 4.8). My mean control mIPSC amplitude was greater than values previously reported in P30 mice (Jiao et al. 2006) and in P28 – 48 old rats (Salin and Prince 1996). However, this discrepancy could be related to both: different recording temperatures and the minimum mIPSCs amplitude threshold used to select the event. In this thesis, the noise threshold was defined as 2.5 times the average root mean square noise (Clements and Bekkers 1997) (calculated from 10 cells) and it was found to be equal to 10.7 pA. The distribution of the raw events in deprived cortex also did not show a significant shift compared to control. Those results suggest that short deprivation does not affect the amplitude of mIPSCs in deprived cortex.

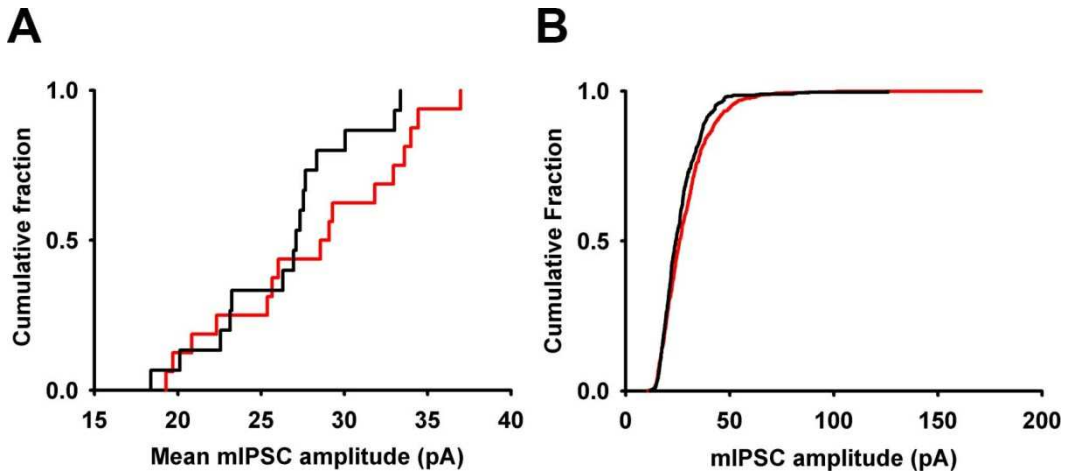

Figure 4.8 Brief deprivation does not affect mIPSC amplitude in layer 2/3 pyramidal cells of deprived cortex

***A**. Cumulative fraction of the distributions of mean mIPSC amplitude in deprived (red, n = 16 neurons) and control (black, n = 15 neurons) cortex. **B**. Cumulative distribution of mIPSC events in control (black, n = 505 events) and in deprived (red, n = 742 events) cortex after 2 - 3 days of whisker trimming.*

4.6.4 mIPSC frequency is increased by brief deprivation

mIPSC frequency was calculated dividing the number of events recorded in a trace by its time - lengths (10 seconds). The median mIPSC frequency in deprived cortex was increased compared with age matched control (control: 2.7 [2.4 – 4.5] Hz, n =16; deprived: 4.3 [3.6 – 5.9] Hz, n = 15; P = 0.03, Kolmogorov-Smirnov test) (Figure 4.9). The frequency recorded in control was lower than the mIPSC frequency reported by others (Salin and Prince 1996, Jiao et al. 2006, Kobayashi et al. 2008). The lower mIPSC frequency, together with the increased mIPSC amplitude, may reflect the different amplitude threshold used to detect the events. Moreover, it was very difficult to increase the membrane resistance above 200 MΩ, even 30 minutes after the break in, when Caesium ions and QX314 should have blocked the voltage gated potassium and sodium channels respectively. Therefore, small distal events might have been lost due to lack of adequate space clamp.

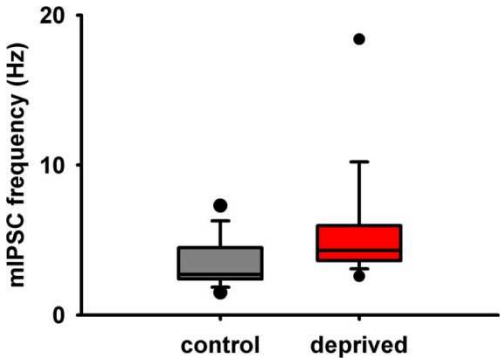

Figure 4.9 Brief deprivation increased mIPSC frequency in pyramidal cells of deprived cortex

Box and whisker plot of mIPSC frequency recorded from layer 2/3 pyramidal neurons in control (dark gray, n = 15 neurons) and in deprived (red, n = 16 neurons) cortex after brief sensory deprivation.

4.6.5 The change in mIPSC frequency is not related to the location of the neuron in the barrel

It has previously been reported that the extent of plastic changes in the excitatory circuits is affected by the distance of the cells from the border between pared and deprived area (Cheetham et al. 2007). Therefore, I investigated whether the change in mIPSC frequency was correlated with the location of the cell in the barrel. I found no correlation between the mIPSC frequency and the location of the cells in respect to the border either in control ($r(8) = 0.31$, $P = 0.450$) or in deprived cortex ($r(14) = -0.04$, $p = 0.884$).

4.6.6 Passive membrane properties

The initial and stabilized membrane potential, membrane resistance, capacitance and time constant of the cells used in the mIPSC analysis are reported in Table 4.2. None of the above passive membrane properties resulted significantly different in deprived cortex compared to control.

Table 4.2 Passive membrane properties of layer 2/3 pyramidal cells are unaffected by deprivation

	V_m Break in (mV)	V_m (mV)	R_m (MΩ)	T_m (ms)	C_m (pF)
Control	-68 (-65 – -70)	-41 (-23 – -44)	218 (187– 261)	64 (54 – 91)	335 (247 – 429)
Deprived	-70 (-66 – -72)	-42 (-29 – -45)	183 (148 – 211)	55 (43 – 63)	297 (252 – 343)
Statistics	p = 0.290 (Mann-Whitney)	p = 0.394 (Mann-Whitney)	p = 0.138 (Mann-Whitney)	p = 0.079 (Mann-Whitney)	p = 0.397 (Mann-Whitney)

Passive membrane properties of layer 2/3 pyramidal cells in control and deprived cortex. Membrane potential at break in (V_m), membrane potential after 30 minutes of intracellular and bath drugs application (V_m), membrane resistance (R_m), time constant (T_m) and membrane capacitance (C_m) were not significantly different between the two groups.

4.7 Chapter summary

In this chapter I investigated whether 2 - 3 days of whiskers trimming affected synaptic transmission by recording mEPSPs in FS interneurons and mIPSCs in pyramidal cells of layer 2/3 in deprived cortex. I found that:

- 2 - 3 days of brief deprivation altered the global excitatory input onto FS interneurons. mEPSP amplitude increased in deprived cortex compared to control. In contrast, the frequency of mEPSP was not affected by brief deprivation.

- brief sensory deprivation also altered the global inhibitory input onto pyramidal cells. mIPSC frequency increased in deprived cortex after sensory deprivation, while mIPSC amplitude was unchanged compared to age matched controls.

4.8 Miniature postsynaptic events discussion

4.8.1 mEPSPs in layer 2/3 FS interneurons

mEPSPs have been previously used to investigate the effect of long period (> 2 weeks) of whiskers trimming on the excitatory circuits of the spared and deprived cortex of adult animals (Cheetham et al. 2007). mEPSPs have also been recorded in layer 2/3 pyramidal cells in deprived and spared cortex after 2 - 3 days of sensory deprivation (Barnes 2010). At this time-point, there is no change in mEPSP amplitude or frequency, but local connectivity in deprived cortex increases from 4% to 12%. The lack of changes in mEPSP frequency and amplitude could be interpreted as the effect of re-allocation of synaptic inputs, with loss of long distance input and increase of local synaptic contacts (Barnes 2010). In this chapter, I showed that altered sensory experience affects the excitatory drive onto FS interneurons. The increased mEPSP amplitude, without changes in frequency, indicates that the synaptic strength of excitatory inputs is increased. This could be attributed to postsynaptic changes in receptor number and/or configuration (i.e. LTP-like mechanisms) or turnover formation of stronger synapses during the deprivation period. Presynaptic increase of synaptic contacts per connection is unlikely, as no change in mEPSP frequency was found. However, changes in the probability of release and the vesicle content of neurotransmitters cannot be ruled out and will require further investigation (see Chapter 5). The increased mEPSP amplitude in FS interneurons, but not in pyramidal cells, in response to the same deprivation protocol also suggests that excitatory inputs are selectively altered based on the identity of the postsynaptic cell (Reyes et al. 1998).

The increased mEPSP amplitude in FS interneurons could also be interpreted as a homeostatic change. Homeostatic synaptic plasticity is defined as scaling of the connections strength in the appropriate direction to contra balance altered neural activity. Basically, if FS interneuron activity were decreased as a result of whisker trimming in deprived cortex, the mEPSP amplitude would be expected to scale up to maintain the

balance. Homeostatic changes have been previously reported both in culture (Turrigiano et al. 1998, Kilman et al. 2002) and in brain slices prepared from visual cortex (Desai et al. 2002, Goel and Lee 2007). However, the entire distribution of mEPSP/Cs in those cases were scaled up or down by a multiplicative factor, with the only exception of older animals (P95). I found that the distribution of the mEPSPs recorded from layer 2/3 in deprived cortex after 2 - 3 days of sensory deprivation was not shifted by a multiplicative factor (Figure 4.4), suggesting that those changes are not homeostatic. However, the mEPSP frequency was inversely correlated with amplitude in deprived cortex (Figure 4.5), and this same relation has been previously found by Desai et al. during homeostatic mEPSC amplitude scaling down in developing cortex. Nevertheless, the negative correlation between amplitude and frequency may not be necessarily related to homeostatic plasticity. The results found in developing visual cortex could be associated with an increase in synapse number between connected neurons, as it has been reported for hippocampal cells in culture (Liu and Tsien 1995). Therefore, based on these considerations, the hypothesis of a homeostatic change cannot be rejected. As layer 2/3 FS interneuron intrinsic excitability was not altered after 2 - 3 days of whiskers trimming in deprived cortex (see Chapter 3), I investigated whether the inhibitory drive on pyramidal cells was decreased as a result of disinhibition.

4.8.2 mIPSC in layer 2/3 pyramidal cells

I recorded mIPSCs from pyramidal cells in control and deprived cortex after 2 - 3 days of whisker trimming. The animal age and the trimming protocol were the same for mEPSP recordings. mIPSC amplitude was not affected by deprivation. In contrast, mIPSC frequency in deprived cortex increased as a result of sensory deprivation. The mIPSC experiments did not show a decrease in inhibition that could justify a possible homeostatic scaling of mEPSPs in FS interneurons. However, mIPSCs are a measure of global excitatory drive on pyramidal cells, which cannot distinguish between local and long distance inputs or be selective for interneuron subtype. Therefore, although there is no evidence of general disinhibtion in layer 2/3 excitatory circuit, I cannot conclude that the

inhibitory to excitatory component of the negative feedback circuit composed of pyramidal cell and FS interneuron is unaffected by altered sensory experience.

4.8.3 Miniature postsynaptic events interpretation

Miniature postsynaptic events are traditionally believed to reflect the release of single vesicles of neurotransmitter. Therefore, the frequency of miniature postsynaptic events and their amplitude have been used as indicative measure of the strength of the overall excitatory (or inhibitory) drive onto a cell. However, the dynamics of vesicles release at the presynaptic terminal is complex. Even though vesicles share the same biochemical structure, they have different functional properties. Vesicles can be clustered into three different pools: a rapid releasable pool (RRP), a reserve pool and a resting pool (Rizzoli and Betz 2005). Although the physical dimension of each pool, as well as their precise localization is still unclear, the vesicles of the RRP are thought to be the closest to the membrane and the first to be released in response to increased intracellular calcium. As the RRP is completely depleted, the reserve pool starts to be released. In contrast, the role of the resting pool is still unknown, as it seems to be rarely released during physiological stimulation. Recent studies seem to support the idea that the resting pool of vesicles is responsible for the spontaneous release that generates miniature postsynaptic events (Sara et al. 2005, Fredj and Burrone 2009). However, other results seem to identify a common pool for both spontaneous and activity-dependent release (Wilhelm et al. 2010, Groemer and Klingauf 2007, Hua et al. 2010). If spontaneous and activity-dependent releases were supported by different pools of vesicles, then changes in miniature event could not necessarily provide information about activity-dependent transmission. Therefore, miniature postsynaptic events must be interpreted with caution. Miniature postsynaptic events have the limitation of being unspecific, as it is impossible to discriminate whether the inputs coming from different layers are evenly responsible for the changes in events amplitude and frequency.

By recording mIPSCs in pyramidal cells it was also impossible to establish whether sensory deprivation affected specifically the synaptic connections made by a specific interneuron subtype. Moreover, voltage clamp recordings are affected by technical limitations such as space clamping (Spruston et al. 1993, Williams and Mitchell 2008) (see Section 2.4.4.1). The age of the animals and the temperature of the recordings made it very difficult to increase the membrane resistance of the cells above 200 MΩ. This implies that membrane depolarisations occurring in dendrites at great distances from the soma were more likely to be undetected. Although the vast majority of FS interneurons in layer 2/3 of SI have basket cell morphology and make synaptic contacts perisomatically (and therefore these events should be captured even in case of poor space clamping) it is not possible to selectively isolate them. For all these reasons, I used miniature postsynaptic event as a first screening approach to investigate whether brief sensory deprivation affects transmission between excitatory and inhibitory cells of deprived cortex.

Despite all the above mentioned limitations, the results I presented in this Chapter showed that at least some excitatory connections onto layer 2/3 FS interneurons in deprived cortex were potentiated following 2 -3 days of deprivation. FS interneurons mediate both feed-forward inhibition form layer 4 and feedback inhibition within layer 2/3. However, the excitatory inputs coming from layer 4 are the ones less likely to be potentiated. In fact, when deprivation starts during the critical period of plasticity and lasts 6 – 12 days, the excitatory connections onto layer 2/3 neurons depress (Allen et al. 2003). Moreover, as a result of deprivation (reduced whisker stimulation), the firing rates measured in vivo with electrode arrays are modestly reduced both in layer 4 and in layer 2/3 (Celikel et al. 2004). If deprivation reduced the excitatory drive from layer 4 onto layer 2/3, this would be reflected in a reduction in layer 2/3 FS interneuron firing and, consequently, a decreased inhibitory drive onto layer 2/3 pyramidal neurons. The inhibitory drive was however unchanged after 2 – 3 days of deprivation while the excitatory dive was potentiated. A possible explanation for these results could involve a form of anti – Hebbian plasticity. According to this hypothesis, layer 2/3 FS interneurons would receive reduced excitatory

inputs from layer 4 following deprivation and therefore, decrease their firing. In absence of firing, the excitatory input coming from active neurons could be selectively strengthened by a form of anti – Hebbian plasticity. The potentiation of these inputs could then balance the reduced activity in layer 4, and maintain the inhibitory drive onto layer 2/3 pyramidal cells.

Before focusing on the possible mechanisms by which the excitatory connections onto layer 2/3 FS interneurons are potentiated, it is important to identify which specific inputs are involved in this process. I reasoned that FS interneurons receive input from excitatory neurons both in layer 4, layer 2/3 and layer 5. As previously mentioned, evidence in the literature suggests that layer 4 inputs are more likely to be depressed rather than potentiated. Because the vast majority of synaptic inputs on layer 2/3 neurons come from local circuits (Dantzker and Callaway 2000), I reasoned that local excitatory inputs onto FS interneurons were a good candidate to undergo synaptic strengthening. Therefore, I further investigated plastic changes in the disynaptic negative feedback circuits in layer 2/3 by performing simultaneous recording of connected pyramidal cell and FS interneurons (see Chapter 5).

Chapter 5 Plasticity of pyramidal cell – FS interneuron unitary connections

5.1 Introduction

In this chapter I investigated the effects of sensory deprivation on the local feedback circuits comprising a pyramidal cell and a FS interneuron in layer 2/3 of primary somatosensory cortex. Whisker trimming started between P30 and P43, when the sensory map in layer 2/3 had already been established (Stern et al. 2001) and lasted for 2 or 3 days. Control animals were also in the P32 – P45 age range of deprived animals.

5.2 Background

Altered sensory experience in adulthood induces cortical map plasticity more prominently in layer 2/3 (Fox 1992). Long periods of sensory deprivation induce shrinking of the representation of the missing input and expansion of the surrounding representations (Buonomano and Merzenich 1998). The effects of long period of sensory deprivation on the connectivity and the synaptic strength of connections within the excitatory circuitry of layer 2/3 have been investigated previously (Cheetham et al. 2007). In particular, a long period of whisker trimming (> two weeks) starting at P19 induces a decrease in connectivity between pyramidal cells in deprived cortex with no changes in strength of the remaining connections. In contrast, connection strength is notably increased in spared cortex while connectivity is unaffected by non traumatic sensory deprivation. To investigate the timeline of connections loss in deprived cortex, previous work in my lab has shown that there is a temporary increase in excitatory connectivity after 2 - 3 days of deprivation, after which connectivity decreases (Barnes 2010).

It has been hypothesized for a long time that the reorganization process in the deprived area could involve a temporary phase of disinhibition, which would allow the excitatory circuitry to rearrange (Jones 1993, Akhtar and Land 1991). However, the role of the inhibitory circuitry in cortical map reorganization is still poorly understood.

As many mechanisms of plasticity are shared between sensory cortical areas (Fox and Wong 2005), studies performed in visual cortex may provide useful insights. In visual cortex, monocular deprivation during the pre-critical period (P15 - P17) strongly reduces the unitary amplitude of IPSC generated by FS interneurons onto star pyramidal neurons in layer 4 (Maffei et al. 2004). Conversely, when the animals undergo monocular deprivation during the critical period of plasticity (P18 - P21) both the excitatory and inhibitory connections between star-pyramid cells and FS interneurons are increased in deprived cortex (Maffei et al. 2006).

This suggests that inhibitory circuits are strongly affected by altered sensory experience during development, but whether they play a key role in adult plasticity is still debated. Recent evidence from imaging studies of gephyrin puncta (markers for inhibitory synapses) shows that monocular deprivation in adulthood induces rapid loss of inhibitory synapses on dendritic spines of layer 2/3 pyramidal cells (van Versendaal et al. 2012). This would support the dishinibition theory. However, this study did not provide any electrophysiological measurement to support the imaging data. Therefore functional evidence still needs to be produced.

In barrel cortex, changes in the feedforward inhibitory circuits have been reported when sensory experience is altered during the critical period of plasticity in layer 2/3 (House et al. 2011). When trimming starts at P12 and lasts for 6 to 12 days, the strength of layer 4 excitatory input onto layer 2/3 FS interneurons is significantly decreased, even more than the excitatory input onto principal cells (House et al. 2011, Allen et al. 2003). Moreover, the inhibitory connections from FS interneurons onto layer 2/3 pyramidal cells are strengthened in the deprived area and the connectivity is also increased. These data, together with the findings from visual cortex, confirm that inhibitory circuits are extremely plastic during map critical periods, but it leaves open the question of whether inhibitory circuit plasticity occurs in mature cortex. A structural study of axon dynamics shows that after 2 days of whisker plucking in adult mice, the axonal arborisations of interneurons in

deprived cortex undergo extended reorganization, with retraction of axons closer to the cell body and extension of axons projecting to the surrounding areas (Marik et al. 2010). Changes in axonal dynamic are a strong clue that inhibitory circuitry may be reorganizing. Accordingly, here, I aim to investigate electrophysiologically whether a brief period of whisker trimming functionally modifies the inhibitory circuits of deprived cortex outside the critical period.

5.3 Method summary

Bilateral trimming of the upper 3 rows (A, B and C plus the outlier α, β and γ) or the lower 2 rows (D and E 2 rows plus the outlier γ and δ) of whiskers was performed daily from P30 - P43 animals for either 2 or 3 days. Control animals were handled daily and were exposed to a sham procedure. On the day of the experiment animals were anesthetized and perfused with dissection ACSF. Brain slices were cut out a 65° angle to the midline (Finnerty et al. 1999), kept in bubbled ACSF at 36℃ for an hour and subsequently at room temperature until recording. Recordings were performed at physiological temperature (37℃) using a K^+ based internal solution (see Section 2.3). Recordings started by establishing a whole-cell patch onto a putative FS interneuron. Once the identity of the cell was confirmed by its firing pattern in response to 500 ms depolarizing current pulses, a second pipette filled with a different dye was used to patch the closer pyramidal cell, identified by morphological features. To test the presence of an excitatory connection, the pyramidal cell was induced to fire action potentials by injecting currents (1.5 - 3.5 nA) in 8 pulses of 2 – 3 ms each at 20 Hz, while the interneuron was recorded in current clamp at resting membrane potential. If the pyramidal cell was connected to it, unitary excitatory postsynaptic potentials (uEPSPs) would be generated in the interneuron and appear as a short latency positive peaks in response to the action potentials elicited in the presynaptic neuron. Subsequently, the cells were tested for the presence of a reciprocal connection. In order to identify uIPSPs the pyramidal cell was held at -50 - -55 mV, while the interneuron was induced to fire action potentials by injecting currents (0.8 - 1.5 nA) in 8 pulses of 2 ms each at 10 Hz.

5.4 Statement of work and work in conjunction with others

During my PhD training I recorded 35 pairs of synaptically connected FS interneurons and pyramidal cells. In some analysis of this chapter, 7 FS interneuron – pyramidal cell control pairs that had been previously recorded in the lab by Dr. Claire Cheetham and Dr. Clarissa Edwards were pooled together with my dataset with their permission in order to increase the power of my statistical analysis.

5.5 Unitary excitatory postsynaptic potentials (uEPSPs) in FS interneurons

The unitary excitatory postsynaptic potential (uEPSP) is the postsynaptic depolarization generated by the synchronous, activity-dependent release of neurotransmitter from the presynaptic terminal of an excitatory cell synaptically coupled to the postsynaptic neuron. uEPSPs from pyramidal cells onto FS interneurons were recorded in layer 2/3 of primary somatosensory cortex in control and deprived cortex after 2 - 3 days of sensory deprivation. At first, a whole-cell patch clamp recording was established from a visually recognized interneuron. Once the cell had been unequivocally identified as FS interneuron, a second recording was established from the closest healthy-looking pyramidal cell. 10 to 50 raw traces of uEPSPs in response to presynaptic trains of 8 action potentials at 20 Hz were acquired and analysed for each connection.

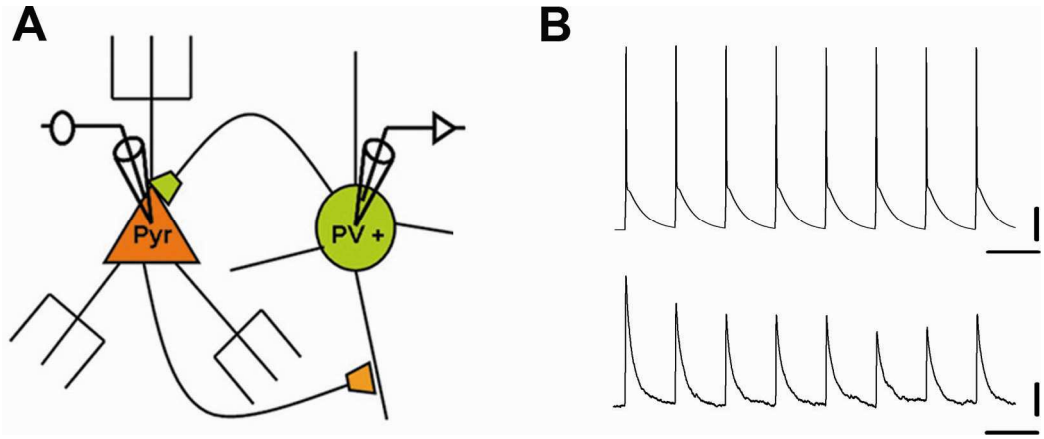

Figure 5.1 uEPSP recorded from layer 2/3 FS interneuron

A. *Schematic of simultaneous dual whole-cell recordings from a pyramidal cell and a FS interneuron.* ***B.*** *A train of 8 action potentials at 20 Hz in the presynaptic pyramidal cell generates 8 short latency uEPSPs in the postsynaptic FS interneuron. uEPSP trace is the average of 50 trials. Scale bars, from top to bottom: 20 mV, 50 ms, 0.5 mV, 50 ms.*

5.5.1 uEPSP Synaptic efficacy

The mean uEPSP amplitude (average of 10 - 50 trials) was used as a measure for the mean synaptic efficacy of each connection. The mean amplitude of the first uEPSP in the train ($uEPSP_1$) was then considered as the baseline synaptic efficacy, while the steady state synaptic efficacy was defined as the mean of the 6^{th}, 7^{th} and 8^{th} uEPSPs ($uEPSP_{6-8}$) in the train (Figure 5.1).

5.5.1.1 Short deprivation did not affect baseline synaptic efficacy of excitatory connections onto FS interneurons

The mean amplitude of $uEPSP_1$ in control was 1.19 [0.83 – 1.73] mV; n = 15 (from 15 rats). There was no significant difference in $uEPSP_1$ mean amplitude between control and deprived cortex (deprived: 1.26 [0.88 – 2.11] mV; n = 19 (from 18 rats); p = 0.627, Mann-Whitney Rank Sum test) (Figure 5.2A). Therefore, I concluded that a short period of whisker trimming did not affect the baseline synaptic efficacy of local excitatory

connections onto layer 2/3 FS interneurons in deprived cortex compared with control cortex.

5.5.1.2 Short deprivation did not affect steady state synaptic efficacy of excitatory connections onto FS interneurons

I then compared the steady state synaptic efficacy in control and deprived cortex after 2 - 3 days of deprivation. I found no difference between the steady state efficacy (mean $uEPSP_{6-8}$) in control cortex and in deprived cortex (control: 0.87 [0.45 – 1.15] mV, n = 15; deprived: 1.02 [0.52 – 2.15] mV, n = 19; p = 0.367, Mann-Whitney Rank Sum test) (Figure 5.2B). Hence, a short period of deprivation did not affect the synaptic efficacy of excitatory connections onto FS interneurons in layer 2/3 of deprived primary somatosensory cortex.

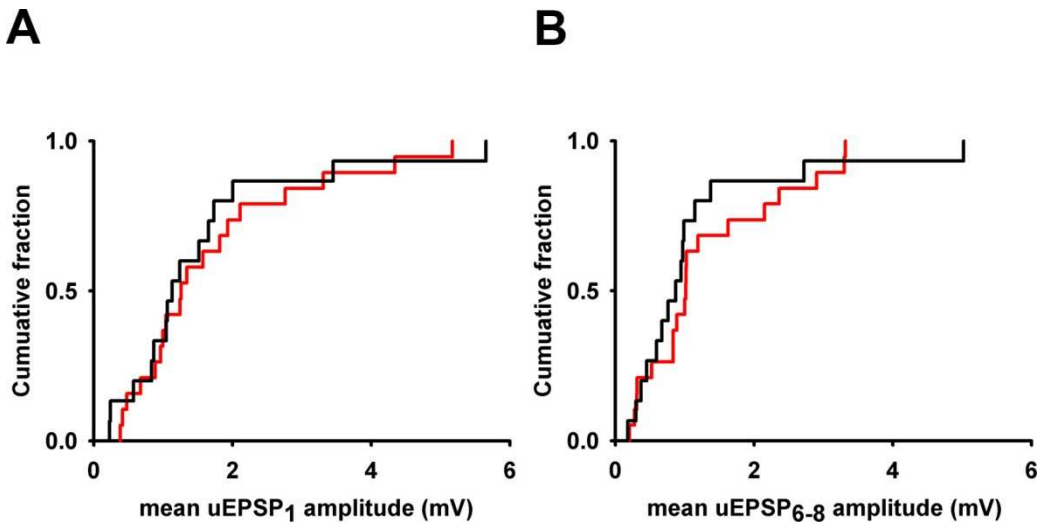

Figure 5.2 Baseline and steady state synaptic efficacy are not affected by deprivation

A. *Cumulative fraction plot of mean baseline uEPSP amplitude in control (black) and deprived (red) cortex.* ***B.*** *Cumulative distributions of mean uEPSP steady state amplitude in control (black) and deprived (red) cortex.*

5.5.2 Short term synaptic dynamics

The synaptic efficacy of a connection is not a constant value, but it is strongly affected by previous activity in a train (Markram and Tsodyks 1996, Thomson 1997, Thomson and Deuchars 1997). As a result of continuous presynaptic firing, the amplitude of the uEPSPs can decrease (depress), increase (facilitate) or show a combination of the two. In neocortex, the short term changes in uEPSP amplitude of excitatory connections onto interneurons have been shown to be presynaptic in origin but dependent on the postsynaptic cell identity (Reyes et al. 1998). In particular, excitatory connections on FS interneurons of neocortical layer 2/3 show depressing behaviour when stimulated in a range of frequencies between 10 and 80 Hz (Reyes et al. 1998).

In my control sample, the mean amplitude of the baseline uEPSP was significantly greater than the mean steady state uEPSP amplitude ($p < 0.001$, paired t-test) (Figure 5.3A). Similarly, the baseline uEPSP amplitude was also greater than the steady state uEPSP amplitude in connections from deprived cortex ($p < 0.001$, $W = 160$, Wilcoxon Signed Rank Test). These results show that excitatory connections onto FS interneurons exhibit depressing behaviour, as it has been previously reported by others (Reyes et al. 1998, Beierlein et al. 2003).

5.5.2.1 Short deprivation did not affect the difference between baseline and steady state synaptic efficacy in deprived cortex

The mean amplitudes of uEPSP in the train in control and deprived cortex are reported in Figure 5.3A. In order to quantify the amount of the depression, the difference between mean baseline and mean steady state uEPSP amplitude was calculated ($\Delta uEPSP = uEPSP_1 - uEPSP_{6\text{-}8}$) and is reported in Figure 5.3B. The range of ΔuEPSP was highly variable both in control (-0.06 – +1.01 mV) and deprived cortex (-0.22 – +2.26 mV) with 2 controls and 3 deprived connections showing facilitation. There was no significant difference between the distributions of ΔuEPSP in control and deprived cortex (control: 0.37 [0.07 – 0.40] mV, n = 15; deprived: 0.21 [0.10 – 0.63] mV, n = 19; p = 0.532, Mann-

Whitney Rank Sum test) despite a trend towards reduced depression in deprived cortex. When the uEPSP amplitude was plotted against the difference in uEPSPs between baseline and steady state amplitude, and a positive correlation was found both for control ($R = 0.58$, $n = 15$, $p = 0.02$, Pearson correlation) and deprived ($R = 0.70$, $n = 19$, $p < 0.001$, Pearson correlation) connections (Figure 5.3C).

5.5.3 Paired-pulse ratio

The paired-pulse ratio (PPR) is defined as the ratio between the amplitude of the second to the first PSP (PSP_2/PSP_1) in a train and it is frequently used to quantify the short term behaviour of a connection (Zucker and Regehr 2002, Branco and Staras 2009). Here I investigated whether deprivation affected the PPR. The PPR was calculated as the ratio of the mean responses: $EPSP_2/EPSP_1$ elicited in response to a train of 8 APs at 20 Hz in the presynaptic pyramidal cell. The PPR in control cortex was 0.87 ± 0.06 ($n = 15$) and it was similar to the PPR in deprived cortex (deprived: 0.88 ± 0.04, $n = 19$; $p = 0.823$, t-test) (Figure 5.3D). Both the PPR and the distributions of ΔuEPSP showed that the synaptic dynamics in deprived cortex are very similar to the synaptic dynamics of control connections. Changes in PPR are usually associated with changes in probability of release at the presyniptic terminals (Zucker and Regehr 2002). Since no significant difference in synaptic efficacy was found between controls and deprived connections both for baseline and steady state activity (see Section 5.5.1.1; 5.5.1.2), the lack of changes in PPR is consistent with previous results.

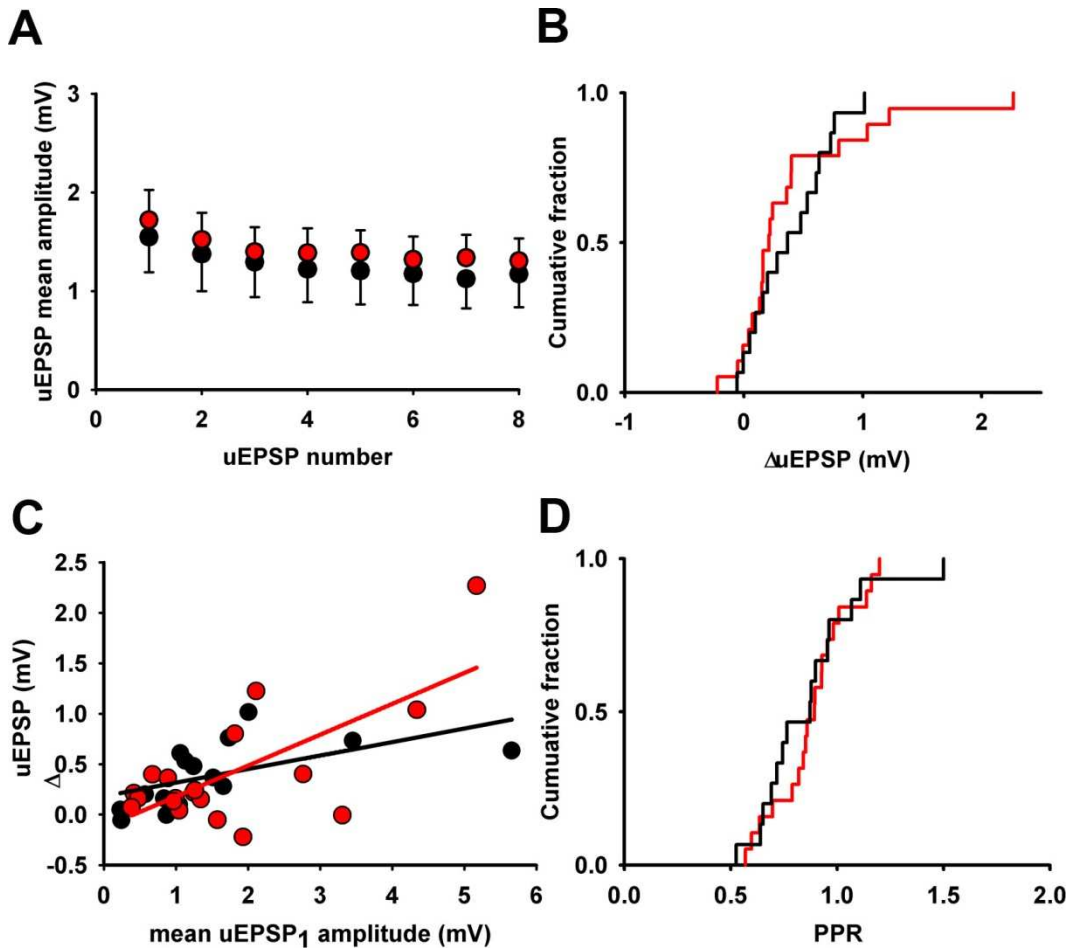

Figure 5.3 The effects of deprivation on synaptic dynamics and PPR

A. *Synaptic dynamics of control (black) and deprived (red) excitatory connections onto FS interneurons in a train at 20 Hz.* ***B.*** *Cumulative fraction plot for difference in uEPSP amplitude between baseline and steady state in control (black) and deprived (red) cortex.* ***C.*** *Scatter plot of mean baseline uEPSP versus ΔEPSP for each connection in control (black) and deprived (red) cortex.* ***D.*** *Cumulative distribution of PPR in control (black) and deprived (red) cortex.*

5.5.4 Reliability of synaptic transmission in Pyramidal to FS interneuron connections

The base of chemical neurotransmission is the synchronous release of neurotransmitter from the presynaptic terminal trigged by the action potential propagation. However, there are situations in which the generation of an action potential in the presynaptic cell does not generate a short latency membrane potential change in the postsynaptic cell. Those situations are referred to as failures. The quantification of failures can be used as a measure of reliability of synaptic transmission for each connection.

5.5.4.1 Short deprivation did not affect the baseline failure rate in connections of deprived cortex

The baseline failure rate was calculated as the ratio between the numbers of $EPSP_1$ failures over the total number of $uEPSP_1$ analyzed (responses and failures) per each connection. The distribution of baseline failure rate for control and deprived cortex is reported in Figure 5.4B. Almost half of the connections (7/15 controls, 10/19 deprived) did not show any $uEPSP_1$ failure. The baseline failure rate for layer 2/3 excitatory connection onto FS interneurons in control cortex was 0.02 [0.00 – 0.12], n = 15. In deprived cortex, the baseline failure rate was found to be similar to control values (deprived failure rate: 0.00 [0.00 – 0.06], n = 19; p = 0.504, Mann-Whitney Rank Sum test). Therefore, short period of deprivation did not affect the baseline failure rate of excitatory transmission onto FS interneurons in deprived cortex. As there was no difference between the two conditions, I pooled the data together. I then plotted baseline efficacy versus baseline failure rate, to test whether the uEPSP amplitude was related to the probability of failure. There was indeed a negative correlation between $uEPSP_1$ amplitude and the failure rate of excitatory connections onto layer 2/3 FS interneurons (C = -0.518, p = 0.002, Pearson product moment correlation) suggesting that connections with bigger responses failed less than connection with smaller responses. An exponential decay curve was best fitted to the data (Figure 5.4D).

5.5.4.2 Short deprivation did not affect the steady state failure rate in connections of deprived cortex

The steady state failure rate was calculated as the ratio between the number of failures in the 6th, 7th and 8th uEPSP of the train over the number of events analysed (failures + responses). The mean steady state failure rate in deprived cortex (0.05 [0.00 – 0.09], n = 19) was not significantly different from the mean steady state failure rate in control cortex (0.07 [0.02 – 0.23], n = 15; p = 0. 345, Mann-Whitney Rank Sum test) (Figure 5.4). The mean steady state failure rate was significantly greater than the mean baseline failure rate in the control group (p = 0.02, paired t-test). Conversely, in the deprived group this difference did not reach statistical significance (p = 0.08, Wilcoxon Signed Rank sum test).

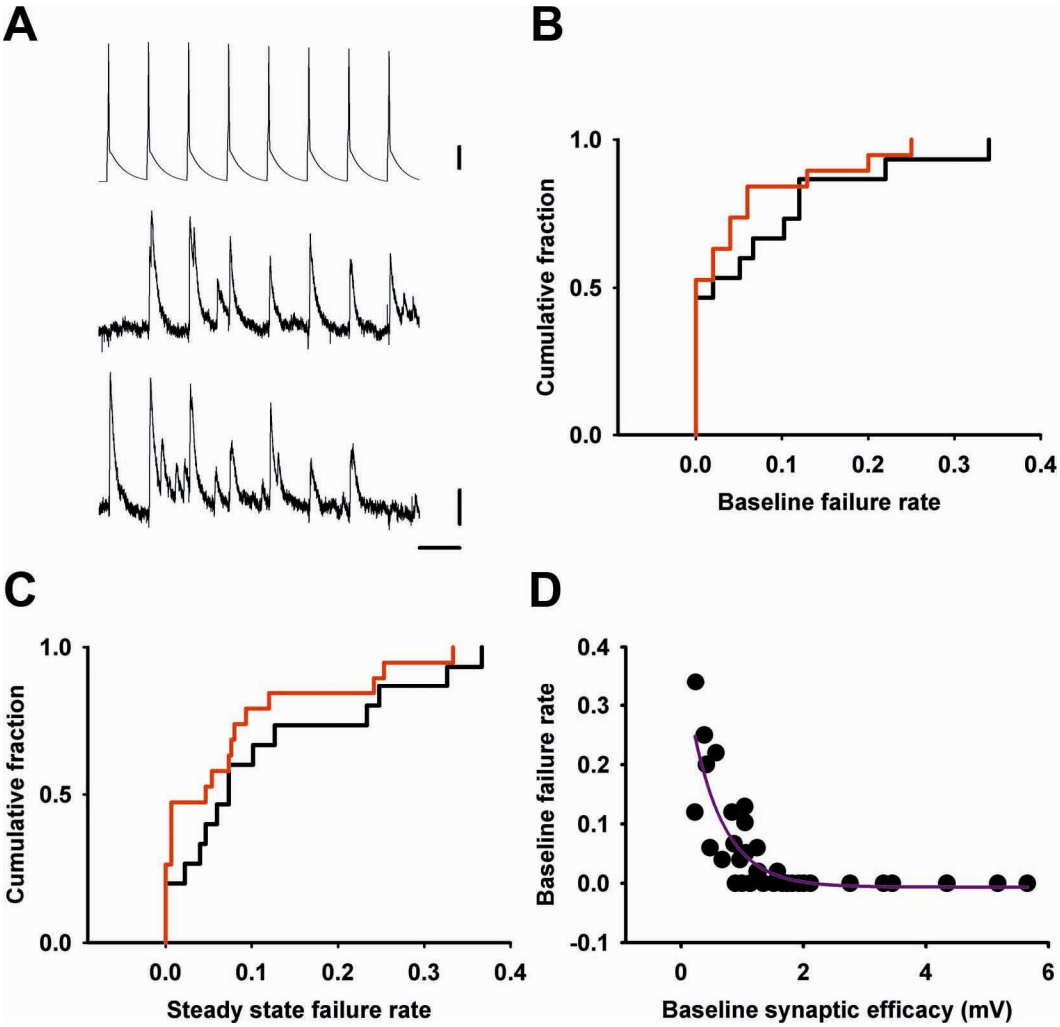

Figure 5.4 Failure rates are not affected by deprivation

A. *Example raw traces of (from top to bottom): a presynaptic train of 8 action potentials at 20 Hz; $uEPSP_1$ failure and $uEPSP_8$ failure.* ***B.*** *Cumulative fraction plot of mean baseline failure rates in control (black) and deprived (red) cortex.* ***C.*** *Cumulative fraction plot of mean steady state failure rates in control (black) and deprived (red) cortex.* ***D.*** *Scatter plot of mean $uEPSP_1$ amplitude versus baseline failure rate in pairs of synaptically connected pyramidal cells - FS interneurons.*

5.5.5 uEPSP synaptic potency

The synaptic efficacy was used in Section 5.5.1 as a measure of synaptic strength. However, the mean uEPSP included failures. Synaptic potency is defined as the strength of the response for each connection, excluding failures. The baseline potency for each connection was calculated by averaging the row measure of all the $uEPSP_1$ excluding failures, for each connection. Likewise, steady state potency was obtained by averaging the row measures of all $uEPSP_{6-8}$ responses without failures.

5.5.5.1 Short deprivation did not affect baseline synaptic potency in deprived cortex

The median baseline potency of excitatory connections onto FS interneurons in control cortex was 1.31 [0.99 – 1.84] mV, n = 15. In deprived cortex the median potency was 1.46 [0.96 – 2.13] mV, n = 19 (Figure 5.5A). Short deprivation did not modify baseline synaptic potency of excitatory connections onto FS interneurons within layer 2/3 in deprived cortex compared with control cortex (p = 0.652, Mann-Whitney Rank Sum test). This result was not unexpected, considering that baseline synaptic efficacy and failure rate were not affected by the deprivation. The cumulative fraction distribution for the baseline synaptic potency in control and deprived cortex is reported in Figure 5.5A. The histogram

distribution of all the raw $uEPSP_1$ from all connections of control and deprived cortex is also reported in figure Figure 5.5C.

5.5.5.2 Short deprivation did not affect steady state synaptic potency in deprived cortex

The median steady state potency in deprived cortex (1.22 [0.67 – 2.23] mV, n = 19) was not different from the steady state potency in control cortex (1.14 [0.78 – 1.49] mV, n = 15; p = 0. 445, Mann-Whitney Rank Sum test) (Figure 5.5B). The steady-state potency was found to be significantly smaller compared with the baseline potency both in the control ($p < 0.001$, paired t-test) and in the deprived group ($p = 0.01$, Wilcoxon Signed Rank sum test).

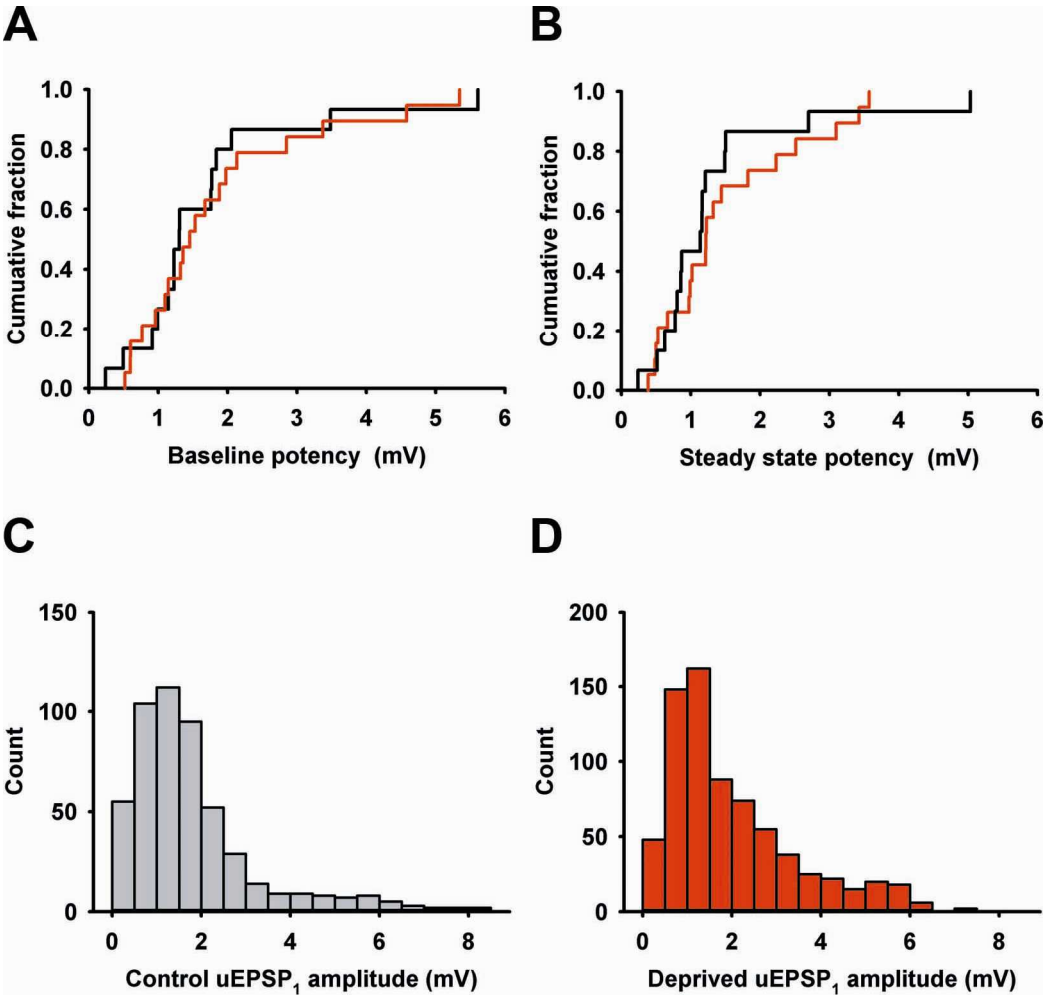

Figure 5.5 Synaptic potency is unaltered by brief deprivation

A. *Cumulative fraction plot of mean baseline potency in control (black) and deprived (red) cortex.* ***B.*** *Cumulative fraction plot of mean steady state potency in control (black) and deprived (red) cortex.* ***C.*** *Histogram of raw uEPSP$_1$ amplitude from all control cortex connections (n = 15).* ***D.*** *Histogram of raw uEPSP1 amplitude from all deprived connections (n = 19).*

5.5.6 uEPSP shape parameters

uEPSP shape was characterized by looking at: latency, 10 – 90% rise time and half width (see Section 2.4.5). The above parameters were measured from raw uEPSPs. Latency was considered as the time between the peak of the action potential in the presynaptic cell and the start of the uEPSP in the postsynaptic interneuron. The 10 – 90% rise time was defined as the time between the 10^{th} and the 90^{th} centiles of the uEPSP rising phase. The half-width was the width of the uEPSP measured at the uEPSP half amplitude. The baseline and steady state uEPSP parameters are reported in Figure 5.6

5.5.6.1 Deprivation did not affect uEPSP latency

The mean baseline uEPSP latency in control cortex was 0.58 ± 0.03 ms, n = 15. In deprived cortex, the mean uEPSP latency was 0.57 ± 0.03 ms, n = 19 (Figure 5.6A). Deprivation did not affect the mean uEPSP baseline latency (p = 0.811, t- test). The steady state latency (the mean of raw uEPSP latency from the 6^{th} to the 8^{th} uEPSPs in the train) was also unaffected by deprivation (control: 0.69 [0.55 – 0.85] ms, n = 15; deprived: 0.66 [0.60 – 0.74] ms, n = 18; p = 0.651, Mann-Whitney Rank sum test) (Figure 5.6B). When compared with the baseline latency, the steady state latency was found to be significantly longer, both in control (p < 0.001, paired t-test) and deprived (p = 0.002, Wilcoxon Signed Rank Sum test).

5.5.6.2 Deprivation did not affect uEPSP 10 – 90% rise time

The median baseline rise time of uEPSP in control cortex was 0.64 [0.49 – 0.75] ms, n = 15, and it was not significantly different from the median uEPSP rise time in deprived cortex (deprived uEPSP rise time: (0.71 [0.57 – 0.86] ms, n = 19; p = 0.445, Mann-Whitney Rank Sum test) (Figure 5.6C). Likewise, steady state rise time was also unaffected by deprivation (control: 0.95 [0.59 – 1.88] ms, n = 15; deprived: 0.95 [0.61 – 1.50] ms, n = 19; p = 0.627, Mann-Whitney Rank Sum test) (Figure 5.6D). However, when compared within the train, there was a significant increase in rise time between steady baseline and steady

state rise time both in control ($p < 0.005$, Wicoxon Signed Rank Sum test) and in deprived ($p = 0.01$, Wicoxon Signed Rank Sum test).

5.5.6.3 Deprivation did not affect uEPSP half-width

The mean baseline uEPSP half-width was 5.38 ± 0.25 ms, n = 15, in control cortex and 5.72 ± 0.38 ms, n = 19, in deprived cortex and it was found to be not significantly different ($p = 0.495$, t-test) (Figure 5.6E). The steady state uEPSP half-width was also similar in control and deprived cortex (control: 5.14 [4.74 – 5.79] ms, n = 15; deprived: 5.37 [4.57 – 5.89] ms, n = 19; $p = 0.945$, Mann-Whitney Rank Sum test) (Figure 5.6F). uEPSPs half-width was also unchanged during the train, as there was no difference between baseline and steady state half-width both in control ($p = 0.783$, paired t-test) and in deprived cortex ($p = 0.152$, paired t-test).

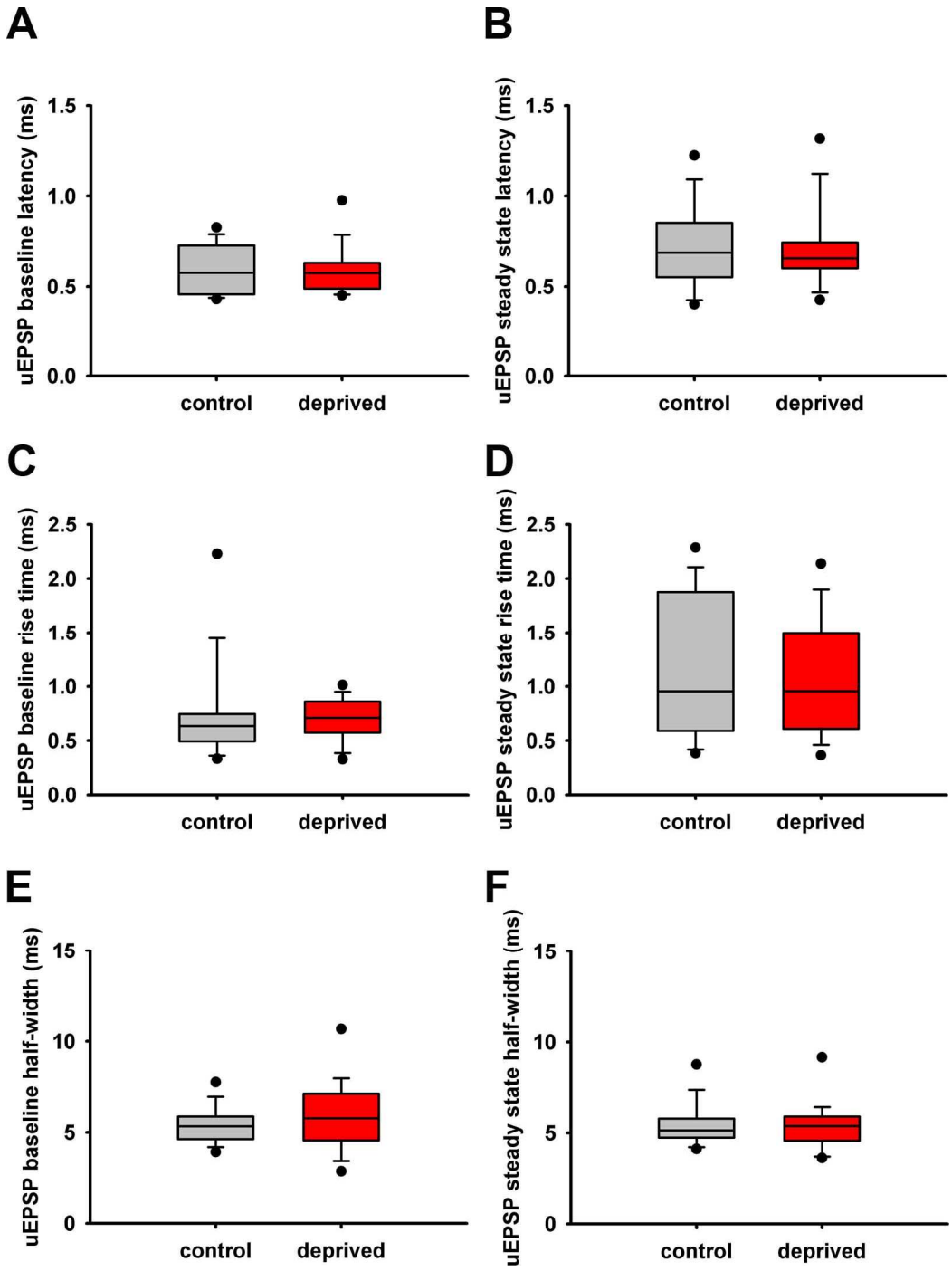

Figure 5.6 uEPSP shape parameters

***A**. Whisker plot of uEPSP baseline latency in control (gray) and deprived (red) cortex.* ***B**. Steady state uEPSP latency in control (gray) and deprived (red) cortex.* ***C**. Whisker plot of uEPSP baseline rise time in control (gray) and deprived (red) cortex.* ***D.** Whisker plot of uEPSP steady state rise time in control (gray) and deprived (red) cortex.* ***E.** Whisker plot of*

uEPSP baseline half-width in control (gray) and deprived (red) cortex. ***F.*** *Steady state uEPSP half-width in control (gray) and deprived (red) cortex.*

5.5.7 Pyramidal cell - FS interneuron connectivity

Connectivity is a measure of how likely a cell makes synaptic contacts onto its neighbouring cells. In particular, I looked at the probability of connections between pyramidal cells and FS interneurons of layer 2/3 in control and deprived cortex. Because FS interneurons are a less common type of cell compared to pyramidal neurons, I first looked for a cell with morphological features of FS interneurons in the right barrel column. Once the whole-cell recording configuration was established and the identity of the cell verified, I then patched the nearest healthy-looking pyramidal cell. The connection was tested by injecting 2 - 3 s current pulses (1-3 nA) in the pyramidal cell while recording the membrane potential of the FS interneuron in current clamp. 10 – 20 trials were averaged in order to detect a connection. If no short latency postsynaptic peak was identified in response to each AP fired in the presynaptic cell, the pyramidal cell was classified as not connected to the FS interneuron. At this point the pipette was removed from that pyramidal cell and the next cell was tested. I tested 34 pairs of pyramidal cell – FS interneurons in control cortex, 20 of which were connected. In deprived cortex, 20 of the 32 pairs tested were found to be connected. These results gave a similar percentage of probability of connection in control (59%) and in deprived cortex (62.5%) ($p = 0.957$, Chi-square test) (Figure 5.7A). Therefore, brief period of whisker trimming did not alter the connectivity of pyramidal cells onto FS interneurons in layer 2/3 compared with control cortex.

5.5.8 Passive membrane properties

The passive membrane properties: membrane capacitance (C_m), membrane resistance (R_m), time constant (τ_m) and membrane potential (V_m) were recorded for both FS interneurons and pyramidal cells. These properties were not altered by the deprivation both in pyramidal cells and in FS interneurons, as reported in Table 5.1.

Table 5.1 Passive membrane properties of FS interneuron and pyramidal cells

	FS interneurons		Pyramidal neurons	
	control	**deprived**	**control**	**deprived**
Capacitance (pF)	97 ± 8	99 ± 9	225 ± 25	237 ± 22
	p = 0.799 (t-test)		p = 0.739 (t-test)	
Resistance (MΩ)	49 [44 – 72]	49 [43 – 60]	40 [33 – 44]	48 [30 – 78]
	p = 0.586 (Rank Sum test)		p = 0.624 (Rank Sum test)	
Time constant (ms)	5.4 ± 0.4	5.0 ± 0.4	10.6 ± 0.8	9.4 ± 0.7
	p = 0.462 (t-test)		p = 0.252 (t-test)	
Membrane potential (mV)	-70 ± 2	-68 ± 1	-71 [-77 – -65]	-75 [-78 – -67]
	p = 0.422 (t-test)		p = 0.434 (t-test)	

5.5.9 Pyramidal cell - FS interneuron pairs location

The location of dye filled cells was recorded using fluorescence microscopy images acquired with a 5X objective. The distance from the pial surface, the distance from the border between spared and deprived columns and the intersomatic distance between pyramidal cell soma and FS interneuron soma were acquired on the x and y dimensions, but they do not account for their location on the z axes. There was no significant difference in distances from pial surface or border between spared and deprived areas for both pyramidal cells and FS interneurons between control and deprived cortex. The measures and statistics are reported in Table 5.2 and Figure 5.7B. There intersomatic distances between connected pairs of neurons were also similar in control ad deprived cortex (Table 5.2).

Table 5.2 FS interneurons and pyramidal cells location

	Control (n = 15)		Deprived (n =19)	
	FS interneuron	**Pyr cell**	**FS interneuron**	**Pyr cell**
Distance from pia (µm)	286 ± 23	266 [208 – 341]	280 ± 11	274 [251 – 300]
Distance from border (µm)	189 ± 40	191 ± 40	225 ± 32	224 ± 29
Intersomatic distance (µm)	31 [17 – 43]		28 [21 – 47]	

There was no difference between control and deprived cortex in either: distance from pia (pyramidal cells: p =0.678, Rank Sum test; FS interneurons: p =0.807, t-test), distance from spared – deprived border (pyramidal cells: p =0.499, t-test; FS interneurons: p =0.478, t-test) or the intersonatic distance between pairs of connected neurons (p = 0.957, Rank Sum test).

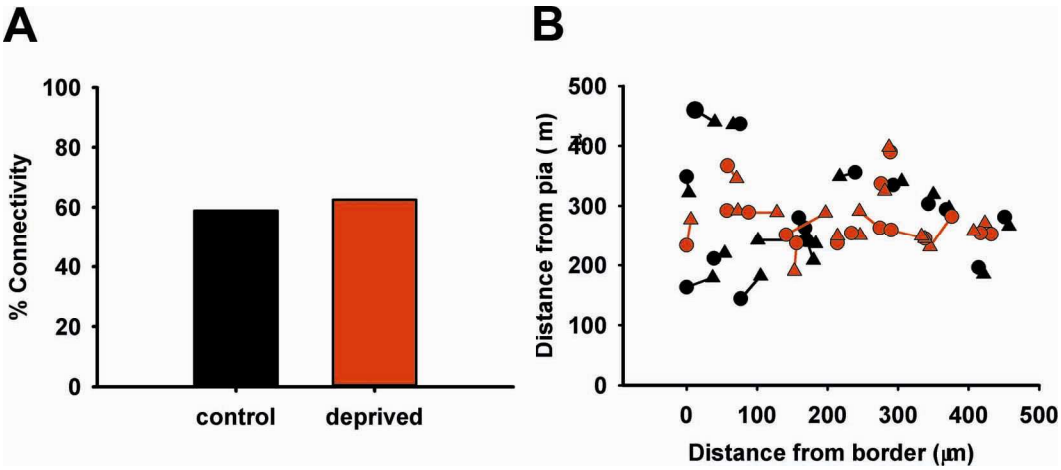

Figure 5.7 Connectivity and pair location

A. *Bar chart of the percentage of synaptically connected pair of pyramidal cells and FS interneurons over the total number of pair tested in control (black) and deprived (red) cortex.* ***B.*** *Scatter plot of connected pair of pyramidal cells (triangles) and FS interneurons (circles). On the x axes is reported the distance from the border between spared and deprived cortex; on the y axes the distance from the pial surface.*

5.5.10 Spike timing dependent plasticity (STDP) of excitatory connections onto L2/3 FS interneurons

Spike timing dependent plasticity was initially discovered in excitatory connection on pyramidal cells (Markram et al. 1997, Bi and Poo 1998). This phenomenon consists in a change in the strength of connection between two cells whose increase or decrease is determined by the temporal relationship between the spikes in the pre- and postsynaptic neurons. In particular, when the presynaptic cell fires an action potential in a windows of approximately 10 milliseconds before the postsynaptic action potential firing (“pre-post”), the connection between the two neurons is strengthened (LTP). When the order is reversed and the presynaptic cell fires an action potential after the postsynaptic neuron (“pre-post”), the connection is weakened (LTD). A recent study has shown that excitatory synapses on inhibitory cells in layer 2/3 of somatosensory cortex exhibit changes in

connection strength as a result of STDP (Lu et al. 2007). Interestingly, the same STDP protocols induces changes in different directions according to subtype of the postsynaptic interneurons (Lu et al. 2007). LTS interneurons followed the same rules as excitatory to excitatory connections while both “pre-post” and “post-pre” STDP protocols induces only LTD in FS interneurons. However, these results were obtained in very young rats (P13 - P16). Here I investigated whether a STDP protocol could produce similar effect in animals aged P30 - P45. The stimulation protocol used was the same indicated by Lu et all 2007; 12 trains of 5 correlated spikes at 20 Hz, with the postsynaptic spikes postponed by 8 ms to the presynaptic train. The amplitude of the connection was assessed by measuring the mean $uEPSP_1$ amplitude (average of 50 trials) in a train of 8 uEPSP at 20 Hz before and after the STDP protocol. Five connections in control cortex and 3 connections in deprived cortex were tested for STDP. The data are presented in Figure 5.8. Three control connections decreased the $uEPSP_1$ amplitude after the STDP protocol, while two increased the mean $uEPSP_1$ amplitude. In deprived cortex, of the three connections tested, two showed increased $uEPSP_1$ amplitude while one increased the amplitude. There was no significant difference in amplitude between pre- and post- $uEPSP_1$ amplitude in the control connections tested (pre- $uEPSP_1$ amplitude: 1.13 ± 0.26 mV; post- $uEPSP_1$ amplitude: 1.08 ± 0.28 mV, n = 5; p = 0.463, paired t-test). No difference was found when the control and the deprived data were pooled together (pre- $uEPSP_1$ amplitude: 1.71 ± 0.35 mV; post- $uEPSP_1$ amplitude: 1.59 ± 0.38 mV, n = 8; p = 0.784, paired t-test). Due to the low number of connections tested, these data must be interpreted cautiously. However, there is no convincing evidence that spike timing dependent depression occurs in the dataset reported here. Whether the change could not be detected because of the small sample or STDP could not be induced because of the different age range used compared to Lu et al. requires further investigation.

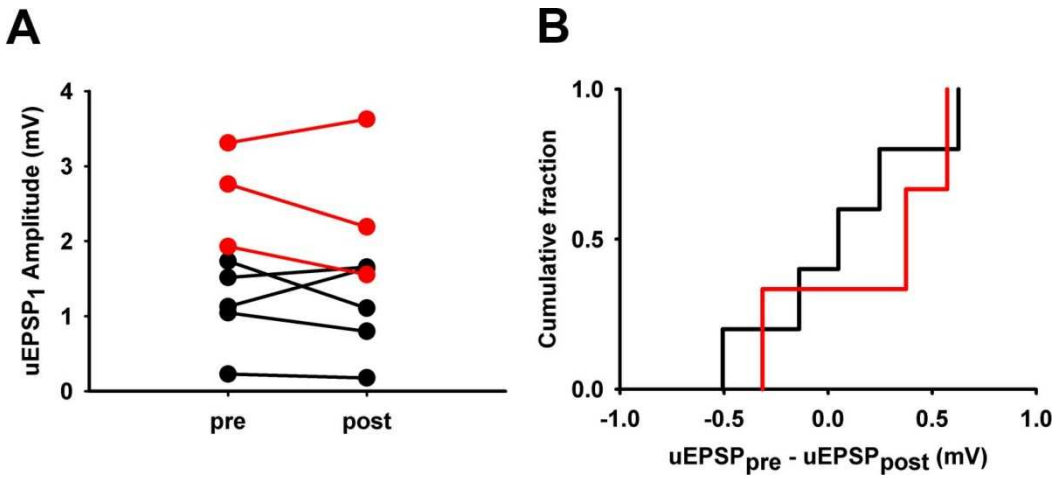

Figure 5.8 Spike timing dependent plasticity of excitatory connections onto FS interneurons

A. *Synaptic efficacy of control (black) and deprived (red) excitatory connections before and after the STDP induction protocol.* ***B.*** *Cumulative fraction plot of difference in uEPSP$_1$ amplitude before and after the STDP induction protocol in control (black) and deprived (red) cortex.*

5.6 Unitary inhibitory postsynaptic potentials (uIPSP) in layer 2/3 pyramidal cells

Unitary inhibitory postsynaptic potentials (uIPSPs) were recorded from 8 pairs of connected FS interneurons and pyramidal cells in layer 2/3 control cortex and 10 pairs in layer 2/3 deprived cortex (Figure 5.9). 8 more control connections, which had been previously recorded by other members of the lab (see Section 5.4) were also included in the control group. uIPSP were recorded with the cell held at -55 mV by current injection. Variation of the holding membrane potential could strongly affect the measure of the uIPSP, therefore the data were considered suitable for analysis only when the membrane potential within each trace and across different trials fluctuated between -54 mV and -57 mV.

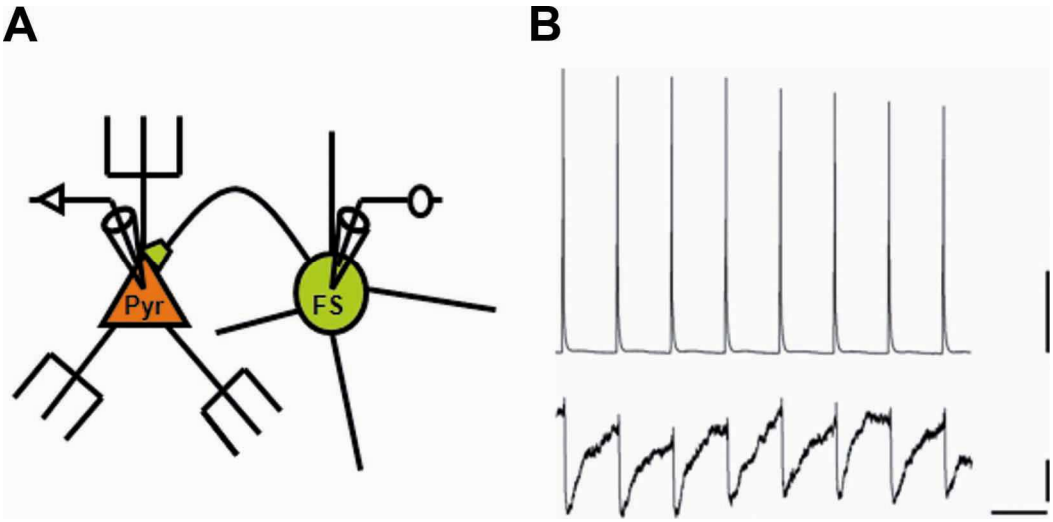

Figure 5.9 uIPSP in layer 2/3 pyramidal cell

A. *Schematic of simultaneous dual whole cell recording from a FS interneuron – pyramidal cell connected pair.* ***B.*** *A train of 8 action potentials at 10 Hz in the presynaptic FS interneuron generates 8 short latency uIPSPs in the postsynaptic pyramidal cell. The membrane potential of the pyramidal cell was hold at -55 mV. uIPSP trace is the average of 50 trials. Scale bars, from top to bottom: 20 mV, 0.1 mV, 100 ms.*

5.6.1 uIPSP reversal potential

The reversal potential for uIPSP generated by FS interneurons onto pyramidal cells was calculated in 3 connections, by holding the cells at different membrane potential and measuring the amplitude of the induced PSP at each potential. In Figure 5.10A the membrane potential of a cell on the x axes is plotted versus the PSP amplitude on the y axes. A polynomial quadratic curve ($f(x) = c + ax + bx^2$) was fitted to the data. By placing $f(x) = 0$, the membrane potential of the postsynaptic cell at which there was no net current flux in response to GABA release was derived. The mean reversal potential for FS inhibitiory connections onto layer 2/3 pyramidal cell was found to be -67.5 ± 1.2 mV. This value was not corrected for liquid junction potential. The reversal potential for inhibitory potentials is similar to the membrane resting potential for pyramidal neurons. Although this

implies that at resting membrane potential the release of GABA does not produce a significant current flux, inhibitory connections do affect the cell by altering its membrane conductance, a phenomenon known as “shunting inhibition” (Mitchell and Silver 2003).

5.6.2 uIPSP synaptic efficacy was not affected by deprivation

The mean $uIPSP_1$ amplitude in pairs of connected FS interneurons and pyramidal cells in control and deprived cortex was used as a measure of synaptic efficacy. The median $uIPSP_1$ amplitude in control cortex was -0.31 [-0.81 - -0.20] mV, n = 16 while the median $uIPSP_1$ amplitude was -0.27 [-0.34 - -0.18], n = 10. The mean $uIPSP_1$ amplitude in deprived cortex showed a trend towards smaller values, but it was not significantly different compared with control (p = 0.280, Mann-Whitney Rank Sum test). The cumulative fraction plot of mean $uIPSP_1$ (absolute values) in control and deprived cortex is reported in Figure 5.10B.

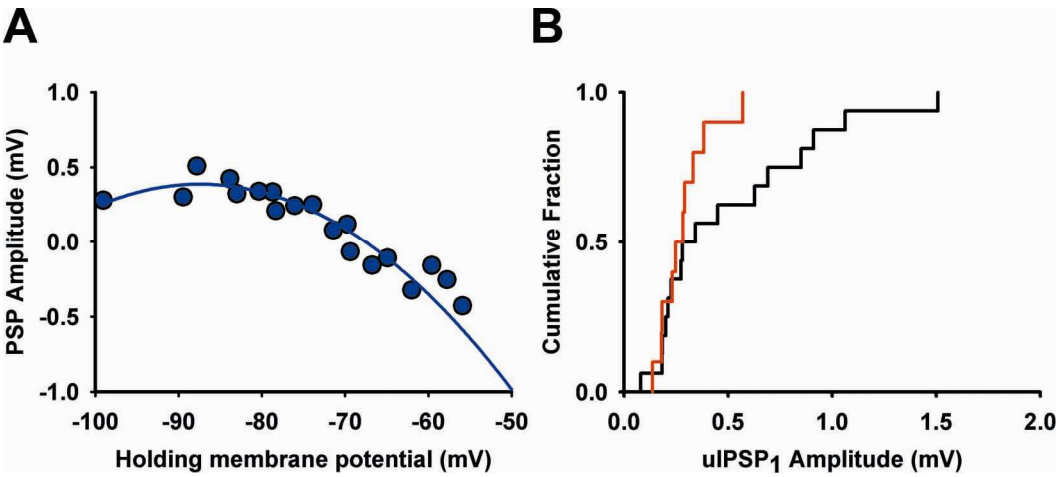

Figure 5.10 uIPSP baseline amplitude is not affected by deprivation

A. *uIPSP reversal potential was calculated by plotting the holding membrane potential of the pyramidal cell versus the amplitude of the PSP generated at each holding potential. The data are from 3 control connections. The polinomial quadratic curve y(x) = -7.08 - 0.17x -10* $^{-3}x^2$ *was fitted to the dataset and used to extrapolate the reversal potential.* ***B.***

Cumulative fraction plot of mean baseline $uIPSP_1$ (absolute values) in control (black) and deprived (red) cortex.

5.6.3 Steady state efficacy and synaptic dynamics

The steady state efficacy, as well as the synaptic dynamics, has been shown to be affected by the frequency at which the action potentials were fired in the presynaptic cell (Beierlein et al. 2003). The vast majority of my data was collected in trains at 10 Hz, whereas data previously collected by other members of the lab were acquired at 20 Hz. The decision to use train at 10 Hz was taken because at 20 Hz the uIPSP overlapped, as their uIPSP decay time was greater than the uEPSP decay time for excitatory connections onto FS interneurons. In order to check whether the stimulation frequency effectively affected the dynamics, I compared a group of control connections where the uIPSP were induced at a frequency of 20 Hz with a second group of control connections where the uIPSP were induced at 10 Hz. Although both the connections stimulated at 10 Hz (baseline: -0.23 ± 0.03 mV; steady state: -0.17 ± 0.02 mV; n = 6, p = 0.014, paired t-test) and the ones stimulated at 20 Hz (baseline: -0.64 ± 0.15 mV; steady state: -0.32 ± 0.07 mV; n = 9, p < 0.005, paired t-test) the steady state efficacy was reduced compared to baseline, the ratios between steady state and baseline efficacy ($uIPSP_{6-8}$/ $uIPSP_1$) were significantly different between the two groups, with bigger depression for the connections stimulated at 20 Hz (10 Hz: 0.70 [0.60 – 0.84], n = 6; 20 Hz: 0.53 [0.45 – 0.54], n = 9; p = 0.04, Mann-Whitney Rank Sum test). This confirmed that it was not possible to merge the two groups (10 and 20 Hz) together. Unfortunately, the deprived group consisted of 6 connections stimulated at 10 Hz and only 2 connections at 20 Hz. Interestingly, the steady state efficacy for both the deprived groups at 10 Hz (baseline: -0.28 ± 0.05 mV; steady state: -0.23 ± 0.04 mV; n = 7) and 20 Hz (baseline: -0.19 ± 0.05 mV; steady state: -0.10 ± 0.04 mV; n = 2),was not reduced compared to the respective baseline (10 Hz: p = 0.132, pared t-test; 20 Hz: p = 0.125, paired t-test). However this evidence is not enough to support a change in dynamics in deprived cortex due to the very low sample of deprived connections stimulated at 20 Hz (n = 2).

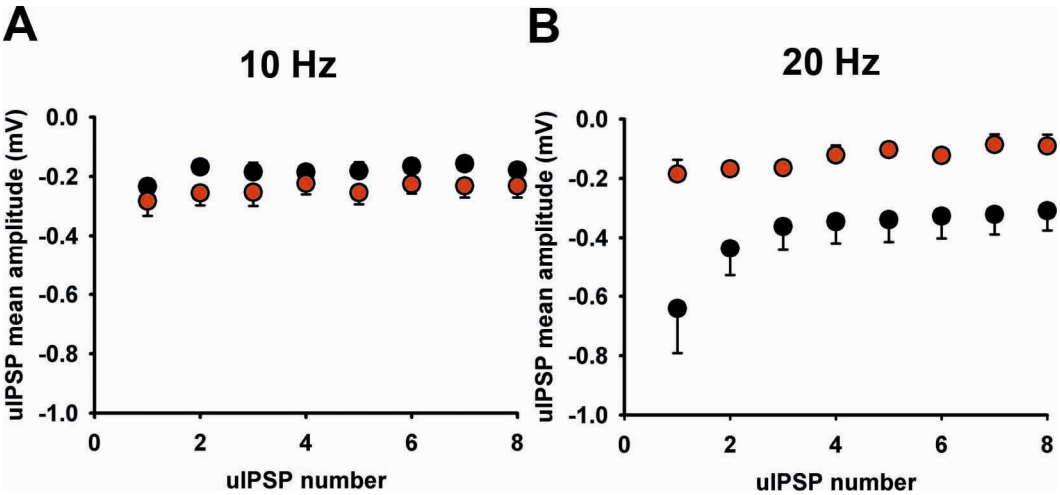

Figure 5.11 uIPSP synaptic dynamics at 10 and 20 Hz

A. *Synaptic dynamics of unitary inhibitory connections in deprived (red) and control (black) at 10 Hz.* ***B.*** *Synaptic dynamics of unitary inhibitory connections in deprived (red) and control (black) at 20 Hz.*

5.6.4 uIPSP shape parameters were unaffected by deprivation

uIPSP shape was characterized by looking at: latency, 10 – 90% rise time and half width. The shape parameters were measured at baseline ($uIPSP_1$) from mean uIPSP (average of 20 – 50 trials). The mean baseline uIPSP latency in deprived cortex (0.58 ± 0.05 ms, n =10) was similar to the mean uIPSP latency in control cortex (0.57 ± 0.03 ms, n =15; p = 0.839, t-test). Deprivation also did not affect the baseline 10 – 90% rise time (control: 4.05 ± 0.43 ms, n = 15; deprived: 4.64 ± 0.65 ms, n = 10; p = 0.448, t-test) or the baseline uIPSP half width (control: 18 [4 - 23] ms, n = 15; deprived: 4 [4 - 22] ms, n = 10; p = 0.570, Mann-Whitney Rank Sum test).

5.6.5 FS interneuron – pyramidal cell connectivity

The presence of an inhibitory connection onto the pyramidal cell was checked after testing for the presence of an excitatory connection onto the FS interneurons. In the vast majority of the experiments, if the excitatory connection was not present, the inhibitory connection onto the pyramidal cell was not tested, in order to maximise the search for excitatory connections. Therefore 23 out of 25 pairs where the FS interneuron was inhibiting the pyramidal cell, the connection was reciprocal, meaning that the pyramidal cell was also exciting the FS interneuron. Occasionally however, the presence of inhibitory connections was tested in absence of an excitatory connection (2 pairs). Therefore, the probability of connectivity of FS interneurons to pyramidal cell that I report here is actually better described as a measure of reciprocity. In control cortex, 13 of 21 (61.9 %) FS interneuron-pyramidal cell pairs were connected. After 2 - 3 days of deprivation, the connectivity between FS interneurons and pyramidal cells was unchanged (62.5%, 10 connected pairs out of 16 tested, $p = 0.760$, Chi-square test) (Figure 5.12A). The probability of connection here reported is lower compared to the 90% reported by Packer et al. using two-photon RuBi-Glutamate uncaging (Packer and Yuste 2011), but similar to the rate reported by Avermann et al, in mouse barrel cortex using simultaneous multiple cells recordings (Avermann et al. 2012). However, there could be an underestimation of inhibitory connectivity in my results caused by the testing strategy (biased towards excitatory connections) as well as the inability in some cases to successfully patch the nearest pyramidal cell to the FS interneuron.

5.6.6 uEPSP versus uIPSP in reciprocally connected pairs of FS interneurons and pyramidal cells

Of the 25 inhibitory connections reported in this chapter, 23 were reciprocally connected. This means that the pyramidal cell was exciting the FS interneuron while the same FS interneuron was also inhibiting back the pyramidal cell, in a complete disynaptic negative feedback circuit. As the excitatory – inhibitory balance is extremely important for the stability of neural circuits, I wondered whether the reciprocal connections of a disynaptic

feedback circuit were also balanced. I therefore investigated whether there was a relationship between the uIPSP and the uEPSP of the feedback circuits by plotting the mean baseline uEPSP amplitude versus the absolute value of the mean baseline uIPSP amplitude for both the control and deprived pairs of reciprocally connected neurons, as shown in Figure 5.12B. There was no correlation between EPSP and IPSP amplitudes in the 14 pairs in control cortex ($r(14) = -0.12$, $p = 679$, Pearson correlation) or in the 9 pairs in deprived cortex ($r(9) = -0.66$, $p = 0.05$, Pearson Correlation).

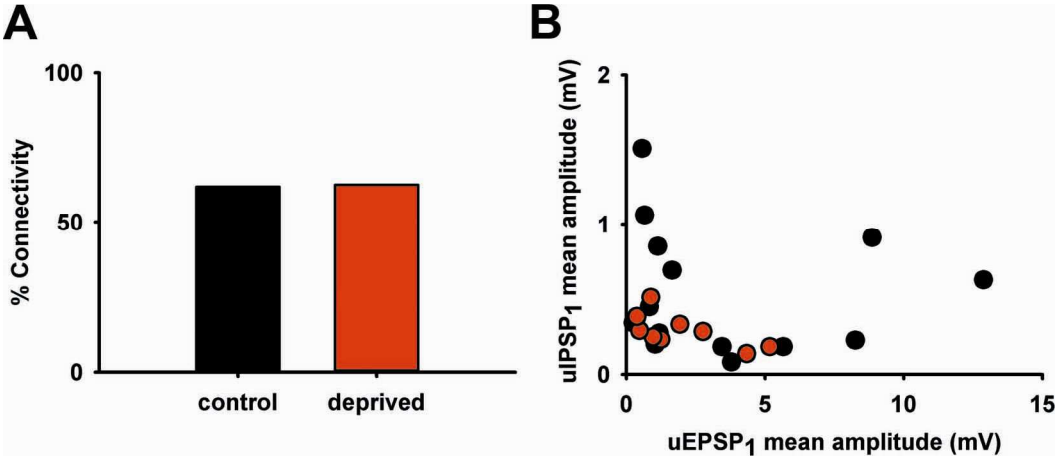

Figure 5.12 Inhibitory connectivity and relationship between uIPSP and uEPSP

A. *Bar charts of percentage of FS interneuron to pyramidal cell connections in control (black) and deprived (red) cortex.* ***B.*** *Scatter plot of mean baseline uEPSP$_1$ amplitude versus mean baseline uIPSP$_1$ amplitude (absolute value) in pairs of reciprocally connected FS interneurons and pyramidal cells in control (black) and deprived (red) cortex.*

5.7 Summary

In this chapter I investigated whether deprivation by whisker trimming functionally altered the feedback circuits comprising a pyramidal cell and a FS interneuron in layer 2/3 of primary somatosensory cortex.

I found that deprivation:

- did not alter: synaptic efficacy, potency, failure rates or uEPSP shape parameters of excitatory connections onto FS interneurons;
- did not alter the synaptic dynamic of excitatory connections on FS interneurons at 20 Hz;
- did not alter the probability of excitatory connections onto FS interneurons;
- did not alter synaptic efficacy or uIPSP shape parameters of FS interneuron inhibitory connections onto pyramidal cells;
- did not alter the probability of connections from FS interneurons onto pyramidal cells.

The results on inhibitory connections here presented have to be interpreted cautiously and considered as preliminary, as a bigger sample is required to elucidate whether efficacy or synaptic dynamics are affected by deprivation.

5.8 Discussion

In this Chapter I investigated whether the increase in mEPSP amplitude that I found in deprived cortex was due to the strengthening of local excitatory input from pyramidal cells of layer 2/3 located within the same barrel. Therefore, I performed simultaneous dual cell recordings from pairs of synaptically connected FS interneurons and pyramidal cells. The evoked unitary EPSP were recorded in response to a train of 8 single AP in the presynaptic pyramidal cell at 20 Hz. I found no difference in the baseline or steady state amplitude of uEPSP between control and deprived cortex.

It has to be noted that the range of baseline average amplitudes for excitatory connections on FS interneurons was extremely wide (0.23 – 5.6 mV). The two populations (control and deprived) were not normally distributed and non - parametric tests were used to compare the two datasets. Moreover, I recorded some very strong connections characterized by

amplitudes greater than 3 times the standard deviation of the population and a probability of release of 1. Connections of this type have been recorded also within the excitatory circuits and referred to as “hub connections” (Barnes 2010). Hub connections are rare but can shift the distributions of the populations and can strongly affect the statistics. The dataset used in this dissertation included 15 pairs of synaptically connected FS interneurons and pyramidal cells in control cortex and 19 pairs in deprived cortex. There was no statistical difference between the two datasets. I feel therefore confident in asserting that a short period of sensory deprivation did not change the strength of excitatory connections onto FS interneurons in deprived barrel cortex. It remains still unknown whether the very large connections (> 5 mV) play specific functions within the network and whether only a particular subset of connections was specifically altered.

I found no change in either paired pulse ratio (PPR) or baseline - steady state amplitude difference between control and deprived cortex. This was not surprising considering that there was no change in synaptic efficacy between control and deprived connections. There was no difference in failure rates between control and deprived connections and the baseline and steady state potencies were also unaffected by deprivation. The distributions of raw uEPSP1 events were similar in control and deprived cortex. Analysis of uEPSP shape parameters such as rise time, latency and half-width also showed no difference between control and deprived connections. These data, together with the lack of change in efficacy and synaptic dynamics, suggest that connections in control and deprived cortex are not different.

Finally, I looked at excitatory connectivity on FS interneurons. It was previously found from my lab that 2 – 3 days of whisker trimming significantly increased the connectivity between excitatory neurons in deprived cortex. In my dataset, I found that the probability of excitatory connections onto FS interneurons in deprived cortex at the same time point of deprivation was not affected by deprivation. This implies that the mechanisms by which inhibitory circuits reorganize following sensory deprivation are different from the ones

active on excitatory circuits. This perhaps is less surprising, considering that excitatory connectivity onto FS interneuron reaches already 60% in normal condition, while connectivity between excitatory cells is about 4%.

The results presented in this Chapter showed that 2 – 3 days of whisker trimming did not potentiate the local excitatory connections onto FS interneurons in layer 2/3 of deprived cortex. The change in mEPSP amplitude that I found in Chapter 4 is therefore not attributable to layer 2/3 local inhibitory feedback circuits. At this time, the origin of those changes is still unknown.

I further investigate inhibitory feedback circuits of layer 2 – 3 by recording uIPSP from pairs of FS interneuron – pyramidal cell that were synaptically connected. In order to record uIPSP, the membrane potential of the pyramidal cell was hold at -55 mV and the inhibitory responses recorded as negative peaks.

The baseline synaptic efficacy in deprived cortex was not significantly different compared with the control group, although there was a trend towards smaller amplitudes in deprived cortex. The data should be interpreted cautiously, as the control group included 16 pairs (8 of which recorded by other people in the lab) while the deprived group consisted of 10 connections. Once again, the non normal distribution of the two populations and the wide range of average amplitudes made it difficult to establish whether a good statistical power was reached. Moreover, if the connection strength had to be decreased, I would expect a very small effect size, probably around 20%, which would be more difficult to prove. In fact, it is well known that even a small decrease in inhibition causes epileptic activity (Chagnac-Amitai and Connors 1989). As the distributions of the two populations seem to be more dissimilar in the fraction of connections with bigger amplitudes, it is possible that deprivation selectively affect a specific population of connections. The trend showed in my data is however opposite to the changes that House *at al.* found by recording pairs of FS –

pyramidal neurons in layer 2/3 of primary somatosensory cortex after 6 – 12 days of deprivation started at P12 (House et al. 2011). The authors reported a significant increase in uIPSP amplitude in developing deprived cortex.

The study of the synaptic dynamic was partially compromised by a difference in the data acquisition protocols. While my data were acquired in response to presynaptic trains of AP at 10 Hz, in order to avoid two events to overlap, the data previously acquired from other members of the lab were recorded in trains at 20 Hz. As the synaptic dynamic change in function of the stimulus frequency, the data could not be merged together.

The inhibitory connectivity onto pyramidal neurons in layer 2/3 was found to be ~ 60% both in control and deprived cortex. The percentage of inhibitory connectivity on pyramidal neurons is very similar to the percentage of excitatory connectivity on FS interneurons. These results further highlight how inhibitory feedback circuits are extensively diffuse in layer 2/3 of primary somatosensory cortex. The percentage of inhibitory connectivity was however biased by the fact that it was tested after excitatory connectivity and its value is therefore more likely to be a measure of reciprocity. In my dataset I did not find a difference in connectivity as a result of deprivation. In contrast, it has been reported that 6 – 12 days of deprivation started at P12 significantly increased the probability of inhibitory connections onto layer 2/3 pyramidal cells from 47% to 79% (House et al. 2011). The degree of plasticity of layer 2/3 inhibitory connections onto pyramidal neurons found by House *et al*. is higher compared with my results. This could find an explanation in the different age of the animals and period of deprivation.

Finally, In This Chapter I also analysed the relationship between uIPSP/ uEPSP in pairs of pyramidal cells – FS interneurons that were reciprocally connected and therefore formed a complete feedback circuits. Interestingly, I did not found a positive correlation as I would have expected. These data imply that the excitatory – inhibitory balance is not maintained at the level of single recurrent feedback circuits.

Chapter 6 Discussion

6.1 Aim of the study

In this thesis, I investigated whether the cortical map reorganization induced by a brief period of altered sensory experience involves long lasting changes to cortical inhibitory circuits in a mature system.

I conducted my investigation in the barrel cortex, the portion of rat primary somatosensory cortex that receives and processes information acquired through the whiskers. Innocuous sensory deprivation was induced by trimming either the upper three or lower two rows of whiskers for 2 – 3 days, starting from P30. Rats aged between P30 and P60 are usually not considered adult, as they have not yet reached sexual maturity. However, the critical period of development for the layer 2/3 cortical map is already completed by P21 (Stern et al. 2001). The synaptic dynamics of excitatory connections are also developed by P28 (Reyes and Sakmann 1999) and the number of inhibitory synapses reaches the adult values by P30 (Micheva and Beaulieu 1996). Similarly, in visual cortex, the density of putative inhibitory synapses reaches its peak at P20 and subsequently decreases whereas the number of synaptic vesicles per terminal of both putative excitatory and inhibitory synapses increases until P28 (Blue and Parnavelas 1983). Therefore, although the rats used were adolescent at the time of the experiment, their barrel cortex maps were mature, and both the excitatory and inhibitory circuits were set in place.

I focused my attention on inhibitory circuits of layer 2/3, which is the principal locus of expression of plasticity after the end of the critical period (Fox 1992). In particular, I looked at local inhibitory feedback circuits comprising a pyramidal cell and a FS interneuron. I chose to investigate this specific subtype of interneuron because their axons synapse on the target cells perisomatically and therefore they are very likely to affect their output. Moreover, FS interneurons have been shown to play a central role in restoring plasticity in adult visual cortex (Pizzorusso et al. 2002). Perineuronal nets are part of the extracellular matrix and are particularly abundant around the somas of FS interneurons. When

chondroitin-sulphate proteoglycans of the extracellular matrix are degraded by injecting the enzyme chondoitrinase, plasticity is restored in adult visual cortex (Pizzorusso et al. 2002). Ocular dominance induced by monocular deprivation during the critical period can also be recovered by chondroitiase injection in adulthood, when coupled with reverse lid-suturing (Pizzorusso et al. 2006). However, a recent study reported that the level of plasticity induced by chondroitinase injection in the contralateral binocular visual cortex of cats that underwent monocular deprivation (from the beginning of the critical period unil 3.5 months) was not sufficient to induce complete functional recovery (Vorobyov et al. 2013).

Previous work in my lab showed that the cortical reorganization induced by altered sensory experience can be visualised in rats by using BOLD – fMRI (Alonso et al. 2008). After three days of whisker trimming, the positive BOLD signal generated by the deflection of a row of spared whiskers in primary somatosensory cortex is enlarged, providing evidence for expansion of spared input onto the deprived area. The expansion is localized in the upper layers (1 – 4) of the neocortex, but the BOLD – fMRI resolution is not great enough to examine the matter further. To investigate whether cortical excitatory circuits were affected at the cellular level, Dr. Samuel Barnes performed electrophysiological recordings from layer 2/3 excitatory neurons in brain slices (Barnes 2010). He found that 2 - 3 days of whisker trimming increased the connectivity between pyramidal neurons of layer 2/3 in the deprived area, from 4 % to 12 %. This increase in connectivity, which occurs contemporaneously to the map expansion imaged with BOLD – fMRI, could represent a mechanism by which cortical circuits rearrange in response to sensory deprivation. However, uncontrolled increase in excitatory feedback loops could lead to excitotoxicity (Chagnac-Amitai and Connors 1989) if not balanced by a change in inhibition.

Although there has been an increased interest in plasticity of the inhibitory circuits in the last decade, its role in mature cortical map reorganization is still unclear. I therefore decided to investigate whether the increase in connectivity between layer 2/3 excitatory pyramidal cells in deprived cortex after 2 – 3 days of whisker trimming is accompanied by

long-lasting changes in layer 2/3 local inhibitory feedback circuits comprising FS interneurons.

6.2 How could sensory deprivation affect inhibition?

6.2.1 The “window of disinhibition” hypothesis

As mentioned above, previous work in the lab found experimental evidence supporting a temporary increase in connectivity between excitatory neurons in deprived cortex after 2 – 3 days of whisker trimming (Barnes 2010). Whether cortical map plasticity also requires modifications of the inhibitory circuits at the same time point remains poorly understood. One hypothesis is that altered sensory experience initiates a period of disinhibition, which enables plasticity of cortical circuitry to occur (Jones 1993, Calford and Tweedale 1991, Garraghty et al. 1991). Increased firing frequencies have been reported in spared cortex of behaving animals after a few hours of deprivation, which it has been suggested to occur as a result of disinhibition (Kelly et al. 1999). To my best knowledge, nobody has yet investigated at the circuit level whether a window of disinhibition could occur in the deprived area.

Multielectrode recordings performed after pairing two whiskers for 3.5 days in adult (~P80) rats show increased neural activity in response to stimulation of spared whiskers in the surrounding deprived area (Lebedev et al. 2000). Those data confirm the expansion of the spared representation imaged using BOLD – fMRI (Alonso et al. 2008). The spatial distribution of activity is instead unchanged in spared cortex when the clipped whiskers are stimulated (Lebedev et al. 2000). Moreover, stimulation of clipped whiskers increases the responses in the surrounding deprived area (Lebedev et al. 2000). These data suggest that sensory deprivation does not just weaken the excitatory connections in the deprived area. The increased responses induced by the stimulation of clipped whiskers in the surrounding deprived area could then be explained by a down-regulation of inhibitory circuits in deprived cortex (Lebedev et al. 2000).

The plastic changes induced by the whisker pairing protocol have recently been investigated by performing *in vivo* whole-cell patch clamp (Gambino and Holtmaat 2012). When the whisker pairing protocol is applied for 2 – 3 days, the spiking in response to stimulation of the principal whisker in spared cortex *in vivo* is not affected (Gambino and Holtmaat 2012). In contrast, the spiking in the spared barrel column induced by the stimulation of the other (surround) spared whisker is significantly increased (Gambino and Holtmaat 2012). Moreover, following deprivation, pairing of the surround whisker stimulation with an AP induces LTP in spared cortex whereas in control animals LTP is induced only by pairing AP with the principal whisker (Gambino and Holtmaat 2012). The deprivation halves the inhibitory conductances evoked by the surrounding whisker in spared cortex, while the excitatory conductances are unchanged (Gambino and Holtmaat 2012). This supports the idea of a tonic disinhibition in response to deprivation, which could be the necessary condition to rearrange layer 2/3 circuits. It remains to be proved whether a similar reduction in inhibition and activation of surround whisker induced STD – LTP occurs in the deprived cortex.

Further evidence in favour of inhibitory circuits' reorganization comes also from structural studies showing axonal rewiring of interneurons in deprived cortex after 2 – 3 days of whisker plucking (Marik et al. 2010). In fact, 2 – 3 days of whisker plucking induces the axons of a subset of interneurons in deprived cortex to extend into the spared area (Marik et al. 2010). Based on this hypothesis, acute disinhibition would allow the excitatory cortical circuits to reorganize, and it would explain both the increase in excitatory connectivity and the expansion of the BOLD signal.

6.2.2 The "neuromodulators - mediated disinhibition" hypothesis

Alternatively, it has been argued that there is transient neuromodulators – mediated disinhibition during active behaviour, which could allow synaptic plasticity of excitatory

connections (Froemke et al. 2007). Experimental evidence in favour of this hypothesis has been reported in auditory cortex (Froemke et al. 2007). Receptive field plasticity in adult primary auditory cortex can be induced when a tone is associated with stimulation of the cholinergic afferents from the nucleus basalis (Kilgard and Merzenich 1998). The mechanisms of cortical field plasticity have been investigated at the cellular level by performing *in vivo* whole-cell patch-clamp recordings from excitatory neurons of primary auditory cortex while pairing a tone with nucleus basalis stimulation (Froemke et al. 2007). After the pairing paradigm, the synaptic inputs in response to the same tone are substantially altered, with facilitation of the excitatory inputs and depression of the inhibitory ones (Froemke et al. 2007). The process of reorganization is extremely rapid and it consists of a sudden (within seconds) reduction of inhibition and a corresponding increase in excitation. The reorganization continues for a few hours and after the initial imbalance, inhibition gradually strengthens to ensure that the balance between excitation and inhibition is preserved (Froemke et al. 2007). Based on these data, the author hypothesises a model in which an initial phase of disinhibition, which allows the strengthening of excitatory inputs stimulus-specific, is followed by a period of general reorganization of excitatory inputs (Froemke and Martins 2011). The last phase of the model requires inhibitory plasticity to restore the excitatory - inhibitory balance (Froemke and Martins 2011).

These two hypotheses make very different predictions about the strength of inhibition during cortical reorganization. In fact, the first hypothesis implies an acute, although not permanent, decrease in inhibition that I would expect to be detected by recording both miniature and unitary IPSC/P. The second hypothesis however, predicts an increase in inhibition to balance the strengthening of excitation. In this case, disinhibition would occur transiently and possibly requite active behaviour. To support this theory, the increase in spiking recorded in spared cortex of behaving animals immediately after whisker trimming was recorded during a screen exploration task, which strongly involved the sensory system but also required attention (Kelly et al. 1999). The final effect predicted by the

neuromodulators hypothesis would however be an increase in inhibition, which should be detected measuring both mIPSC and uIPSP. Nevertheless, it remains also possible that the selective strengthening of some connections could be masked by the depression of other inputs. Whisker trimming, in fact, has been shown to change the behaviour of population of neurons in response to the spared whisker *in vivo* (Margolis et al. 2012). In specific, cells that were more active decreased their activity after 2 days of deprivation and vice versa. If weakening of some excitatory connection resulted in balanced weakening of inhibition, the changes could have remained undetected.

6.3 Summary of findings

I investigated three different aspects of the effect of whisker deprivation on layer 2/3 feedback inhibitory circuits comprising a pyramidal cell and a FS interneuron: intrinsic excitability; excitatory drive on FS interneurons; and inhibitory drive on pyramidal cells. In the following sections each different part of this circuit will be extensively discussed and put into the context of what is already known and compared with other literature. A final conclusion of my findings and how they could fit in the context of the changes occurring on the excitatory circuits will also be discussed at a later stage. Here, however, I would like to summarise briefly the results of Chapters: 3, 4, and 5.

In Chapter 3 I considered whether 2 – 3 days of sensory deprivation affected the passive membrane properties and the intrinsic excitability of FS interneurons in deprived cortex. I found that FS interneurons passive membrane properties were unaltered. Similarly, the parameters that describe a train of action potentials (such as threshold, inter – spike interval, AP height and latency) were unaffected by deprivation. Whisker deprivation did decrease the after hyperpolarisation phase (AHP) amplitude in FS interneurons of deprived cortex and increased their action potential half width. Even so, the firing of FS interneurons in deprived cortex was not significantly altered by deprivation, as the input – output functions in control and deprived cortices were similar.

In Chapter 4 I started to investigate the excitatory drive on FS interneurons by recording miniature EPSP in control and deprived cortex. I found that the overall excitatory drive on FS interneurons in deprived cortex was increased. There was an increase in mEPSP amplitude, with no changes in frequency suggesting that the change could originate postsynaptically. In this Chapter I also recorded mIPSC from pyramidal cells of layer 2/3. The global inhibitory drive on pyramidal cells was also affected by the deprivation. The frequency of mIPSC was increased in deprived cortex, while the amplitude was unaltered. The deprivation - induced changes in both mEPSP amplitude in FS interneurons and mIPSP frequency in pyramidal cells indicated that circuits in layer 2/3 were adapting to the sensory change. However, miniature postsynaptic events reflect global changes in input and do not provide information about which pathways are affected. As the vast majority of input onto layer 2/3 neurons comes from local neurons, I focused my attention on inhibitory feedback circuits.

In Chapter 5 I investigated inhibitory feedback circuits in layer 2/3 comprising FS interneurons and pyramidal cells in deprived and control cortex. I simultaneous recorded from pairs of synaptically connected pyramidal cells and FS interneurons. uEPSP parameters in control and deprived cortex were similar. Connectivity was also unaffected by deprivation. These results imply that a short period of sensory deprivation does not affect the strength of excitatory connection onto FS interneurons. Similarly, I recorded uIPSP in pairs of synaptically connected FS interneurons - pyramidal cells. My preliminary results showed a trend towards decreased mIPSP amplitude in deprived cortex, but they did not reach statistical significance.

6.4 Changes in the layer 2/3 inhibitory feedback circuits of deprived cortex

6.4.1 Changes in intrinsic excitability

Both short (2 – 3 days) or long (> 15 days) period of whisker trimming do not alter intrinsic excitability of pyramidal cells in layer 2/3 of deprived barrel cortex (Cheetham et al. 2007, Barnes 2010). My results in Chapter 3 showed that intrinsic excitability (the input – output curve) is not affected by 2 – 3 days of sensory deprivation. There is however a significant decrease in AHP amplitude and an increase in AP half-width in deprived cortex, which are both consistent with changes in the expression of Kv3 channels (Erisir et al. 1999). However, pharmacological inactivation of Kv3.2 and Kv3.3 channels results also in changes in interspike interval, which I did not find. It could be possible that the change in channel density at this time point is not great enough to compromise the recovery of Na^+ channel from the inactive state. I did not perform recordings of FS interneurons intrinsic excitability after longer period of trimming. However, no changes in intrinsic excitability were found after 2 weeks of trimming when it started at P19 (Edwards 2008). These results make the hypothesis of a change in intrinsic excitability less likely. At least, it would confine it to occur after 3 – 4 days of trimming and disappear before the 15^{th} day of whisker trimming. As Kv3 channels are also expressed at the synapse they may play a role in keeping the AP narrow, limiting the influx of calcium and therefore altering the probability of release (Goldberg et al. 2005). A decrease in density of Kv3 channels could then be responsible for the increase of AP half-width at the presynaptic terminal, which in turn would increase the release probability. The results of Chapter 3 suggest that, if there was a window of disinhibition in layer 2/3 at this time point, it would not be mediated by changes in intrinsic excitability of FS interneurons.

6.4.2 Changes in miniature events

In Chapter 4 I investigated whether sensory deprivation altered the global input onto layer 2/3 neurons by recordings miniature events. Miniature events give us a picture of the overall excitatory or inhibitory drive onto a cell. Although it is not possible to determine

which synaptic events come from a specific layer or cell subtype, miniature events can still be of value in probing whether changes are occurring within a circuit. The mEPSP recorded from FS interneurons in deprived cortex after 2 – 3 days of whisker trimming showed increased amplitudes (see Section 4.5.2). I interpreted the change in mEPSP amplitude as an indicator of circuits' reorganization. However, in the hypothesis of a period of disinhibition, the increase of excitatory drive onto layer 2/3 FS interneurons in deprived cortex is counterintuitive, as increased excitation would lead FS interneurons to be more active. It is possible that these changes reflect a homeostatic process, if the synaptic activity of FS intereneurons had been drastically reduced. The homeostatic scaling reported in literature is however a multiplicative process, while my change does not conform to it (see Section 4.5.4). Alternatively, it is also possible that the increased excitatory drive onto FS interneurons in deprived cortex reflects the anti – Hebbian strengthening of specific connections. In absence of sensory activity, feed forward excitation from layer 4 on FS interneurons is reduced. The strengthening of specific input would then result from the coupling of pre-synaptic excitatory activity with post-synaptic lack of firing in the FS interneuron. Finally, the increase in excitatory drive onto FS interneurons could be a way to strengthen inhibition in response to the increase in local excitatory connectivity that has been shown to occur at this time point (Barnes 2010). Based on these results, I reasoned that, as interneurons act locally and a great portion of inputs that they receive are also local, the most probable sites of change were the local excitatory connections made by pyramidal neurons within the same layer and barrel column.

As I described in Section 6.2.1, one of the most accredited hypotheses on the mechanisms of cortical map plasticity involves disinhibition. As there is evidence that inhibitory connections can also undergo synaptic plasticity (see Section 1.5.2.4), I reasoned that depression of inhibitory connections onto layer 2/3 pyramidal neurons could be a possible mechanism to temporarily reduce inhibition. However, when I probed for changes in inhibitory drive onto pyramidal neurons of layer 2/3 by recording mIPSC in pyramidal cells,

I found no change in mIPSC amplitude. In contrast, the frequency of these events was slightly but significantly increased in deprived cortex. The increase in mIPSC frequency is thought to be associated with a presynaptic change. Yet, it is not possible to establish from my data whether the increase in frequency is attributable to a change in probability of release or increase in the number of synapses. Surprisingly, the mIPSC data recorded from pyramidal neurons do not indicate that inhibition is decreased in deprived cortex. Hence, these results would stand against the hypothesis of a long - lasting window of disinhibition. Nonetheless, miniature events cannot be input - specific for either layer or cell type. Therefore, it is still possible that changes occurring to input coming from layer 2/3 FS interneurons are masked by changes in other inputs.

6.4.3 Changes in evoked responses

In Chapter 5 I showed that excitatory connections onto FS interneurons in deprived cortex are not different from connections in control animals. The original hypothesis that local excitatory connections in deprived cortex underwent synaptic strengthening following deprivation was then not confirmed by my data. This also implies that layer 2/3 excitatory inputs are not responsible for the increased mEPSP amplitude reported in Chapter 4.

When I looked at the strength of inhibitory connections, I also found no change in synaptic efficacy, although there was a trend towards smaller amplitudes in deprived cortex. The trend showed in my data leaves open the possibility of disihnibition, which would be mediated by depression of inhibitory connections onto pyramidal cells. However, this trend did not reach statistical significance, and more experiments are required to validate this hypothesis. Moreover, this trend is opposite to the changes that House *at al.* found by recording pairs of FS – pyramidal neurons in layer 2/3 of primary somatosensory cortex after 6 – 12 days of deprivation started at P12 (House et al. 2011). The authors reported a significant increase in uIPSP amplitude in developing deprived cortex. Yet, these differences could be related both to the different age of the animals and the length of the period of trimming.

Connectivity between pyramidal cells and FS interneurons in layer 2/3 was also unaltered by 2 – 3 days of deprivation. Similarly, the inhibitory connectivity onto pyramidal neurons in layer 2/3 was also found to be ~ 60% both in control and deprived cortex. This implies that, if there is deprivation-induced disinhibition, it is not mediated by a loss of connections between FS interneurons and pyramidal cells.

6.5 Which excitatory inputs onto FS interneurons are strengthened by 2 – 3 days of deprivation?

The results of Chapter 5 proved that the strength of excitatory inputs from local layer 2/3 pyramidal neurons onto FS interneurons is unaffected by deprivation. However, in Chapter 4, I showed that mEPSPs amplitude is increased in FS interneurons of layer 2/3. This implies that some excitatory inputs are potentiated by 2 - 3 days of whisker trimming and leaves the question of which inputs are potentiated, since I have now ruled out local excitatory connections.

Experiments of laser scanning photostimulation in primary visual cortex showed that layer 2/3 FS interneurons receive large excitatory inputs from both layer 4 and layer 5a (Dantzker and Callaway 2000). Therefore, it is possible that the excitatory inputs that strengthen are the ones from layer 4, as FS interneurons mediate both the excitatory feedback and feed forward inhibition. I did not investigate feed forward inhibition in this thesis, but it has been reported that sensory deprivation during the critical period (P12 – 24) reduces excitatory input from layer 4 both onto pyramidal neurons and onto FS interneurons in layer 2/3 (House et al. 2011). Thus, it seems unlikely that shorter period of deprivation started after the end of the critical period could induce an opposite effect. Nonetheless, monocular deprivation induces opposite changes in the inhibitory circuits of visual cortex before and after the critical period (Maffei et al. 2006, Maffei et al. 2004). Therefore, the hypothesis of a change in excitatory input from layer 4 cannot be ruled out.

A second possibility is that layer 5 is the source of strengthened excitatory inputs. Although layer 5, together with layer 2/3, shows experience-dependent plasticity after the end of the critical period, very little is known about how circuits are modified in this layer. However, a recent study has shown that 10 – 14 days of deprivation started at P30 differentially affects the excitatory inputs from layer 2/3 pyramidal cells onto regular spiking and intrinsic bursting cells of layer 5b (Jacob et al. 2012). Interestingly, while layer 2/3 excitatory inputs onto regular spiking neurons from the same deprived column depress after 10 days of trimming, layer 2/3 excitatory inputs onto intrinsic bursting cells from surrounding spared columns are potentiated after just 3 – 5 days (Jacob et al. 2012). Therefore, it would be very interesting to investigate whether the top – down inputs from layer 5 to layer 2/3 also undergo synaptic potentiation and if those inputs are the source of the change in mEPSP amplitude I reported in Chapter 4.

Finally, it is also possible that changes occurred at excitatory cross - barrel connections, which were not investigated. Reorganization of excitatory axons invading the deprived area from the surroundings spared barrel columns has been shown to occur only after ~15 days of deprivation (Marik et al. 2010). However, this does not prevent the possibility for existing connections to undergo synaptic plasticity after just 2 – 3 days of trimming.

6.6 Is there enough evidence for disinhibition?

The lack of changes in the strength or probability of inhibitory connections made by FS interneurons onto pyramidal cells in layer 2/3 of deprived cortex suggests that 2 – 3 days of deprivation does not reduce inhibition. Although the uIPSP results have to be considered preliminary, because of the low power of the statistics, the mIPSP recording also showed no change in amplitude. In contrast, there was a small but significant increase in mIPSP frequency, which would indicate an increase, rather than decrease, in inhibition onto layer 2/3 pyramidal cells.

Nevertheless, it is possible that I could not detect the window of disinhibition because it had occurred at an earlier time point. Increased firing after whisker trimming in spared cortex has been detected within a few hours of whisker trimming (Kelly et al. 1999). Conversely, there is no evidence of increased activity in spared cortex after 2 – 3 days of deprivation, as the BOLD signal expands but does not increase. The axonal arborizations of layer 2/3 interneurons in deprived cortex have been shown to reorganize after 2 – 3 of whisker plucking in adult mice; therefore it is very surprisingly that I could not detect any functional change at the same time point.

However, it is also possible that disinhibition occurs, but it is mediated by a different class of interneurons. Somatostatin-positive interneurons, for example, have been shown to play a fundamental role in the process of lateral suppression in behaving animals (Adesnik et al. 2012). The importance of the role of somatostatin positive interneurons has also been shown in hippocampus (Lovett-Barron et al. 2012). In fact, firing of pyramidal cells in CA1 is increased by silencing somatostatin positive interneurons much more than by silencing parvalbumin – expressing interneurons (Lovett-Barron et al. 2012). This suggests that somatostatin positive cells are the main regulators of the input – output curve of pyramidal neurons in hyppocampal circuits (Lovett-Barron et al. 2012). Moreover, interneurons expressing 5HTR3a receptors could also been involved in the process of cortical maps reorganization. These interneurons do not express parvalbumin or somatostatin and, in layer 2/3 of primary somatosensory cortex, they account for half of the whole interneuron population (Rudy et al. 2011). Chronic administration of the antidepressant fluoxetine, which selectively inhibit serotonin uptake, has been shown to increase the levels of plasticity in adult visual cortex (Maya-Vetencourt et al. 2008). Since extracellular basal levels of GABA are reduced in the visual cortex of fluoxetine-treated adult rats, it is very likely that serotonin affects plasticity by modulating GABAergic transmission (Maya-Vetencourt et al. 2008). It is however unclear whether fluoxetine acts specifically on interneurons expressing 5HTR3a receptors. In fact, a recent study has reported increased

excitability of FS interneurons in prefrontal cortex upon application of fluoxetine, which was dose dependent and mediated by 5HT2 receptors (Zhong and Yan 2011).

As my data do not prove the existence of a window of disinhibition, more investigations are required to test weather cortical map reorganization occurs through transient neuromodulation. Cholinergic modulation, in specific, is very likely to affect plasticity. The main source of cholinergic innervations to the cortex is the nucleus basalis in the basal forebrain (Picciotto et al. 2012). Cholinergic innervations to the cortex are particularly abundant in layer 5 and layer 2/3 (Sachdev et al. 1998). Interestingly, these are the two major locus of experience-dependent plasticity in adult animals (Fox 1992). In barrel cortex, when the cholinergic fibers are destructed by injecting in vivo an immunotoxin specific for cholinergic neurons in the forebrain, the plasticity of the surroundings responses induced by 7 days of whisker pairing paradigm no longer occurs (Sachdev et al. 1998). This strongly supports the idea that reorganization of the deprived areas requires cholinergic modulation. It would be extremely useful to understand how cholinergic modulation affects the cortical circuits during plasticity. However, the study is complicated by the fact that acetylcholine has many different effects in neocortex, pre- and post-synaptically, and it acts by binding both muscarinic and nicotinic receptors in excitatory and inhibitory cells (Picciotto et al. 2012). The types of plasticity induced by acetylcholine are therefore dependent on the cell target and the types and sub-types of receptors involved (Picciotto et al. 2012). A recent study by Nunez et al. investigated in vitro the effects of local administration of acetylcholine on pyramidal cells of layer 5 barrel cortex (Nunez et al. 2012). They found increased excitability of pyramidal cells and enhanced excitatory transmission, while synaptic inhibition was reduced (Nunez et al. 2012). These findings also support the idea that transient disinhibition could be induced by altering neuromodulation. Hence, some useful information could be acquired by investigating whether acetylcholine or acetylcholinesterase inhibitors administration to brain slices has different effects onto layer 2/3 local circuits of control and deprived cortex. The study of cholinergic neuromodulation is however complicated by the fact that its release is tightly

link to behavioural states, attention and learning and memory (Picciotto et al. 2012). Therefore, an exhaustive investigation of how acetylcholine affects the excitatory and inhibitory circuits of primary somatosensory cortex during cortical map plasticity would have to be carried out *in vivo.*

6.7 Conclusions

The lack of significant changes in uEPSP onto FS interneurons and uIPSP onto pyramidal cells suggests that local inhibitory feedback circuits of layer 2/3 in deprived cortex are not affected by 2 – 3 days of whisker trimming. Feedback inhibition is therefore preserved in deprived cortex, while excitatory connectivity transiently increases. This implies that for a period of time, local circuitry in deprived layer 2/3 is unbalanced, as inhibition has not increased to balance excitation. However this condition does not last long, as excitatory connectivity decreases after 4 – 10 days of whisker trimming and the existing excitatory connections on pyramidal neurons weaken. It has been extensively shown that inhibition is extremely important in information processing, both in anesthetised (Wehr and Zador 2003) and awake animals (Crochet et al. 2011, Haider et al. 2013). In a recent study it has been shown how inhibitory responses dominate the excitatory ones in response to visual stimuli in awake animals (Haider et al. 2013). Then, inhibition plays a fundamental role in limiting neural activity both spatially and temporally. It is indeed well established that a small reduction in inhibition leads to dangerous excitotoxicity (Chagnac-Amitai and Connors 1989). However the effects of decreased inhibition are not necessary comparable with increased excitatory connectivity. It is possible that the increase in excitatory connections in deprived cortex does not generate an increase in activity sufficient to require a rebalance of inhibition. Although after 2 – 3 days of whisker trimming the probability of connection between pyramidal neurons in deprived cortex increases from 4% to 12%, the deprived area is subjected to reduced input and therefore reduced neural activity. Moreover, the activity of pyramidal cells in layer 2/3 is very small even in controls, due to sparse coding. Finally, the inputs from layer 4 have also been shown to depress (Allen et al. 2003).

It remains to be established whether the trend towards reduced uIPSP amplitudes in deprived cortex could reach statistical significance. In this case scenario there would be supporting evidence for the existence of a window of disinhibition. This hypothesis however does not find any support from the miniature data, as mIPSP amplitude in pyramidal neurons of deprived cortex was not reduced.

6.8 Future considerations

The results presented in my thesis showed that deprivation affect some excitatory inputs onto layer 2/3 FS interneurons in deprived cortex, but which input are strengthened is still unknown. Future work will then require elucidating this matter further. One possible experiment could involve the use of retrograde tracers to identify the sources of inputs to these interneurons. To test the hypothesis that cross – barrel connections are responsible for the change in mEPSP amplitude, a set of experiments using extracellular stimulation form neighbouring barrels could be performed. Similarly, extracellular stimulation could also be used to investigate the role of connections form layer 5.

As discussed in the previous section, it would be important to perform more experiments to probe whether effectively the strength of uIPSP onto layer 2/3 pyramidal neurons of deprived cortex in not affected by deprivation. If this had to be the case, we would have to assume that the hypothesis of a window of disinhibition in deprived cortex is not supported by the data, at least for FS interneurons. It could be still possible that a different class of interneurons was responsible for cortical disinhibition. If, in contrast, the trend towards reduced uIPSP in deprived cortex was found to be significant, then the hypothesis of a window of disinhibition would find experimental evidence in its favour. This would open a long series of potential experiments. It could be possible, for example, to test whether reduction in FS interneuron activity was sufficient to induce reorganization by selectively combining BOLD FMRI with optogenetic techniques. For example, it would be interesting

to test whether the BOLD signal expansion was reproducible by selective inactivating a subset of FS interneurons expressing halorhodopsin or potassium channels. If indeed disinhibition was able to enhance cortical reorganization, than it would be possible to attempt to increase plasticity pharmacologically in those medical conditions that require reorganization of neural circuits.

References

Adelman, W. J., Jr. and Senft, J. P. (1966) 'Voltage clamp studies on the effect of internal cesium ion on sodium and potassium currents in the squid giant axon', *J Gen Physiol,* 50(2), 279-93.

Adesnik, H., Bruns, W., Taniguchi, H., Huang, Z. J. and Scanziani, M. (2012) 'A neural circuit for spatial summation in visual cortex', *Nature,* 490(7419), 226-31.

Akhtar, N. D. and Land, P. W. (1991) 'Activity-dependent regulation of glutamic acid decarboxylase in the rat barrel cortex: effects of neonatal versus adult sensory deprivation', *J.Comp Neurol.,* 307(2), 200-213.

Albieri, G. and Finnerty, G. T. (2012) 'Neocortical learning, memory and experience-dependent plasticity' in Giese, K. P., ed. *Memory Mechanisms in Health and Disease*, World Scientific Publishing.

Allen, C. B., Celikel, T. and Feldman, D. E. (2003) 'Long-term depression induced by sensory deprivation during cortical map plasticity in vivo', *Nat.Neurosci.,* 6(3), 291-299.

Alloway, K. D., Zhang, M. and Chakrabarti, S. (2004) 'Septal columns in rodent barrel cortex: functional circuits for modulating whisking behavior', *J Comp Neurol,* 480(3), 299-309.

Alonso, B. D., Lowe, A. S., Dear, J. P., Lee, K. C., Williams, S. C. and Finnerty, G. T. (2008) 'Sensory inputs from whisking movements modify cortical whisker maps visualized with functional magnetic resonance imaging', *Cerebral Cortex,* 18, 1314-1325.

Alonso, B. D., Lowe, A. S., Dear, J. P., Lee, K. S. and Finnerty, G. T. (2007) 'Experience-dependent plasticity of rat whisker cortical maps imaged with BOLD fMRI', *Proc.Intl.Soc.Mag.Reson.Med.,* 15, 264.

Ascoli, G. A., Alonso-Nanclares, L., Anderson, S. A., Barrionuevo, G., Benavides-Piccione, R., Burkhalter, A., Buzsaki, G., Cauli, B., Defelipe, J., Fairen, A., Feldmeyer, D., Fishell, G., Fregnac, Y., Freund, T. F., Gardner, D., Gardner, E. P., Goldberg, J. H., Helmstaedter, M., Hestrin, S., Karube, F., Kisvarday, Z. F., Lambolez, B., Lewis, D. A., Marin, O., Markram, H., Munoz, A., Packer, A., Petersen, C. C., Rockland, K. S., Rossier, J., Rudy, B., Somogyi, P., Staiger, J. F., Tamas, G., Thomson, A. M., Toledo-Rodriguez, M., Wang, Y., West, D. C. and Yuste, R. (2008) 'Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex', *Nat Rev Neurosci,* 9(7), 557-68.

Atwood, H. L. and Karunanithi, S. (2002) 'Diversification of synaptic strength: presynaptic elements', *Nat.Rev.Neurosci.,* 3(7), 497-516.

Avermann, M., Tomm, C., Mateo, C., Gerstner, W. and Petersen, C. C. (2012) 'Microcircuits of excitatory and inhibitory neurons in layer 2/3 of mouse barrel cortex', *J Neurophysiol,* 107(11), 3116-34.

Barnes, S. J. (2010) *The cellular mechanisms of experience-dependent plasticity in mature neocortex deprived of sensory input*, unpublished thesis King's College London.

Barnes, S. J. and Finnerty, G. T. (2010) 'Sensory Experience and Cortical Rewiring', *Neuroscientist,* 16(2), 186-198.

Barth, A. L., Gerkin, R. C. and Dean, K. L. (2004) 'Alteration of neuronal firing properties after in vivo experience in a FosGFP transgenic mouse', *Journal of Neuroscience,* 24(29), 6466-6475.

Beaulieu, C. (1993) 'Numerical data on neocortical neurons in adult rat, with special reference to the GABA population', *Brain Res.,* 609(1-2), 284-292.

Beierlein, M., Gibson, J. R. and Connors, B. W. (2003) 'Two dynamically distinct inhibitory networks in layer 4 of the neocortex', *J.Neurophysiol.,* 90(5), 2987-3000.

Bender, K. J., Allen, C. B., Bender, V. A. and Feldman, D. E. (2006) 'Synaptic basis for whisker deprivation-induced synaptic depression in rat somatosensory cortex', *J.Neurosci.,* 26(16), 4155-4165.

Benshalom, G. and White, E. L. (1986) 'Quantification of thalamocortical synapses with spiny stellate neurons in layer IV of mouse somatosensory cortex', *J Comp Neurol,* 253(3), 303-14.

Bi, G. Q. and Poo, M. M. (1998) 'Synaptic modifications in cultured hippocampal neurons: dependence on spike timing, synaptic strength, and postsynaptic cell type', *J Neurosci,* 18(24), 10464-72.

Blue, M. E. and Parnavelas, J. G. (1983) 'The formation and maturation of synapses in the visual cortex of the rat. II. Quantitative analysis', *J.Neurocytol.,* 12(4), 697-712.

Branco, T. and Staras, K. (2009) 'The probability of neurotransmitter release: variability and feedback control at single synapses', *Nat Rev Neurosci,* 10(5), 373-83.

Brecht, M. and Sakmann, B. (2002) 'Dynamic representation of whisker deflection by synaptic potentials in spiny stellate and pyramidal cells in the barrels and septa of layer 4 rat somatosensory cortex', *J.Physiol,* 543(Pt 1), 49-70.

Brumberg, J. C., Pinto, D. J. and Simons, D. J. (1996) 'Spatial gradients and inhibitory summation in the rat whisker barrel system', *J.Neurophysiol.,* 76(1), 130-140.

Bruno, R. M. and Sakmann, B. (2006) 'Cortex is driven by weak but synchronously active thalamocortical synapses', *Science,* 312(5780), 1622-1627.

Bruno, R. M. and Simons, D. J. (2002) 'Feedforward mechanisms of excitatory and inhibitory cortical receptive fields', *Journal of Neuroscience,* 22(24), 10966-10975.

Buonomano, D. V. and Merzenich, M. M. (1998) 'Cortical plasticity: from synapses to maps', *Annu.Rev.Neurosci.,* 21, 149-186.

Bureau, I., von Saint Paul, F. and Svoboda, K. (2006) 'Interdigitated paralemniscal and lemniscal pathways in the mouse barrel cortex', *PLoS Biol,* 4(12), e382.

Calford, M. B. and Tweedale, R. (1988) 'Immediate and chronic changes in responses of somatosensory cortex in adult flying-fox after digit amputation', *Nature,* 332, 446-448.

Calford, M. B. and Tweedale, R. (1991) 'Immediate expansion of receptive fields of neurons in area 3b of macaque monkeys after digit denervation', *Somatosens Mot Res,* 8(3), 249-60.

Castillo, P. E., Chiu, C. Q. and Carroll, R. C. (2011) 'Long-term plasticity at inhibitory synapses', *Curr Opin Neurobiol,* 21(2), 328-38.

Castro-Alamancos, M. A., Donoghue, J. P. and Connors, B. W. (1995) 'Different forms of synaptic plasticity in somatosensory and motor areas of the neocortex', *J.Neurosci.,* 15(7 Pt 2), 5324-5333.

Cauli, B., Audinat, E., Lambolez, B., Angulo, M. C., Ropert, N., Tsuzuki, K., Hestrin, S. and Rossier, J. (1997) 'Molecular and physiological diversity of cortical nonpyramidal cells', *J.Neurosci.,* 17, 3894-3906.

Celikel, T., Szostak, V. A. and Feldman, D. E. (2004) 'Modulation of spike timing by sensory deprivation during induction of cortical map plasticity', *Nat.Neurosci.,* 7(5), 534-541.

Chagnac-Amitai, Y. and Connors, B. W. (1989) 'Horizontal spread of synchronized activity in neocortex and its control by GABA-mediated inhibition', *J.Neurophysiol.,* 61(4), 747-758.

Chakrabarti, S. and Alloway, K. D. (2006) 'Differential origin of projections from SI barrel cortex to the whisker representations in SII and MI', *J Comp Neurol,* 498(5), 624-36.

Cheetham, C. E., Barnes, S. J., Albieri, G., Knott, G. W. and Finnerty, G. T. (2012) 'Pansynaptic Enlargement at Adult Cortical Connections Strengthened by Experience', *Cereb Cortex*.

Cheetham, C. E., Hammond, M. S., Edwards, C. E. and Finnerty, G. T. (2007) 'Sensory experience alters cortical connectivity and synaptic function site specifically', *J.Neurosci.,* 27(13), 3456-3465.

Cheetham, C. E., Hammond, M. S., McFarlane, R. and Finnerty, G. T. (2008) 'Altered sensory experience induces targeted rewiring of local excitatory connections in mature neocortex', *J.Neurosci.,* 28(37), 9249-9260.

Chen, H. X., Jiang, M., Akakin, D. and Roper, S. N. (2009) 'Long-term potentiation of excitatory synapses on neocortical somatostatin-expressing interneurons', *J Neurophysiol,* 102(6), 3251-9.

Chen, J. L., Flanders, G. H., Lee, W. C., Lin, W. C. and Nedivi, E. (2011) 'Inhibitory dendrite dynamics as a general feature of the adult cortical microcircuit', *J Neurosci,* 31(35), 12437-43.

Chiaia, N. L., Fish, S. E., Bauer, W. R., Bennett-Clarke, C. A. and Rhoades, R. W. (1992) 'Postnatal blockade of cortical activity by tetrodotoxin does not disrupt the formation of vibrissa-related patterns in the rat's somatosensory cortex', *Brain Res Dev Brain Res,* 66(2), 244-50.

Chiaia, N. L., Rhoades, R. W., Fish, S. E. and Killackey, H. P. (1991) 'Thalamic processing of vibrissal information in the rat: II. Morphological and functional properties of medial ventral posterior nucleus and posterior nucleus neurons', *J Comp Neurol,* 314(2), 217-36.

Chow, A., Erisir, A., Farb, C., Nadal, M. S., Ozaita, A., Lau, D., Welker, E. and Rudy, B. (1999) 'K(+) channel expression distinguishes subpopulations of parvalbumin- and somatostatin-containing neocortical interneurons', *J Neurosci,* 19(21), 9332-45.

Clements, J. D. and Bekkers, J. M. (1997) 'Detection of spontaneous synaptic events with an optimally scaled template', *Biophys.J.,* 73(1), 220-229.

Connors, B. W. and Gutnick, M. J. (1990) 'Intrinsic firing patterns of diverse neocortical neurons', *Trends Neurosci.,* 13, 99-104.

Connors, B. W., Gutnick, M. J. and Prince, D. A. (1982) 'Electrophysiological properties of neocortical neurons in vitro', *J.Neurophysiol.,* 48(6), 1302-1320.

Crochet, S., Poulet, J. F., Kremer, Y. and Petersen, C. C. (2011) 'Synaptic mechanisms underlying sparse coding of active touch', *Neuron,* 69(6), 1160-75.

Cudmore, R. H. and Turrigiano, G. G. (2004) 'Long-term potentiation of intrinsic excitability in LV visual cortical neurons', *J Neurophysiol,* 92(1), 341-8.

Dan, Y. and Poo, M. M. (2004) 'Spike timing-dependent plasticity of neural circuits', *Neuron,* 44(1), 23-30.

Dantzker, J. L. and Callaway, E. M. (2000) 'Laminar sources of synaptic input to cortical inhibitory interneurons and pyramidal neurons', *Nat Neurosci,* 3(7), 701-707.

Desai, N. S., Cudmore, R. H., Nelson, S. B. and Turrigiano, G. G. (2002) 'Critical periods for experience-dependent synaptic scaling in visual cortex', *Nat.Neurosci.,* 5(8), 783-789.

Desai, N. S., Rutherford, L. C. and Turrigiano, G. G. (1999a) 'BDNF regulates the intrinsic excitability of cortical neurons', *Learn Mem,* 6(3), 284-91.

Desai, N. S., Rutherford, L. C. and Turrigiano, G. G. (1999b) 'Plasticity in the intrinsic excitability of cortical pyramidal neurons', *Nat.Neurosci.,* 2(6), 515-520.

Diamond, M. E., Armstrong-James, M., Budway, M. J. and Ebner, F. F. (1992) 'Somatic sensory responses in the rostral sector of the posterior group (POm) and in the ventral posterior medial nucleus (VPM) of the rat thalamus: dependence on the barrel field cortex', *J Comp Neurol,* 319(1), 66-84.

Diamond, M. E., Huang, W. and Ebner, F. F. (1994) 'Laminar comparison of somatosensory cortical plasticity', *Science,* 265, 1885-1888.

Diamond, M. E., von Heimendahl, M. and Arabzadeh, E. (2008a) 'Whisker-mediated texture discrimination', *PLoS.Biol.,* 6(8), e220.

Diamond, M. E., von Heimendahl, M., Knutsen, P. M., Kleinfeld, D. and Ahissar, E. (2008b) ''Where' and 'what' in the whisker sensorimotor system', *Nat.Rev.Neurosci.,* 9(8), 601-612.

Disterhoft, J. F., Coulter, D. A. and Alkon, D. L. (1986) 'Conditioning-specific membrane changes of rabbit hippocampal neurons measured in vitro', *Proc Natl Acad Sci U S A,* 83(8), 2733-7.

Edwards, C. E. J. (2008) *The role of inhibition in layer 2/3 cortical map reorganisation*, unpublished thesis King's College London.

Elbert, T., Flor, H., Birbaumer, N., Knecht, S., Hampson, S., Larbig, W. and Taub, E. (1994) 'Extensive reorganization of the somatosensory cortex in adult humans after nervous system injury', *Neuroreport,* 5(18), 2593-7.

Erisir, A., Lau, D., Rudy, B. and Leonard, C. S. (1999) 'Function of specific K(+) channels in sustained high-frequency firing of fast-spiking neocortical interneurons', *J Neurophysiol,* 82(5), 2476-89.

Fagiolini, M. and Hensch, T. K. (2000) 'Inhibitory threshold for critical-period activation in primary visual cortex', *Nature,* 404(6774), 183-186.

Fatt, P. and Katz, B. (1952) 'Spontaneous subthreshold activity at motor nerve endings', *J.Physiol.(Lond.),* 117(1), 109-128.

Feldman, D. E. (2012) 'The spike-timing dependence of plasticity', *Neuron,* 75(4), 556-71.

Feldman, D. E. and Brecht, M. (2005) 'Map plasticty in somatosensory cortex', *Science,* 310 810-815.

Feldmeyer, D. (2012) 'Excitatory neuronal connectivity in the barrel cortex', *Front Neuroanat,* 6, 24.

Feldmeyer, D., Egger, V., L□bke, J. and Sakmann, B. (1999) 'Reliable synaptic connections between pairs of excitatory layer 4 neurones within a single 'barrel' of developing rat somatosensory cortex', *J.Physiol.(Lond.),* 521, 169-190.

Feldmeyer, D., L□bke, J., Silver, R. A. and Sakmann, B. (2002) 'Synaptic connections between layer 4 spiny neurone-layer 2/3 pyramidal cell pairs in juvenile rat barrel cortex: physiology and anatomy of interlaminar signalling within a cortical column', *J.Physiol.(Lond.),* 538(Pt 3), 803-822.

Feldmeyer, D., Lubke, J. and Sakmann, B. (2006) 'Efficacy and connectivity of intracolumnar pairs of layer 2/3 pyramidal cells in the barrel cortex of juvenile rats', *J.Physiol,* 575(Pt 2), 583-602.

Finnerty, G. T., Roberts, L. S. E. and Connors, B. W. (1999) 'Sensory experience modifies short-term dynamics of neocortical synapses', *Nature,* 400, 367-371.

Fino, E., Packer, A. M. and Yuste, R. (2012) 'The Logic of Inhibitory Connectivity in the Neocortex', *Neuroscientist*.

Fino, E. and Yuste, R. (2011) 'Dense inhibitory connectivity in neocortex', *Neuron,* 69(6), 1188-203.

Foeller, E., Celikel, T. and Feldman, D. E. (2005) 'Inhibitory sharpening of receptive fields contributes to whisker map plasticity in rat somatosensory cortex', *J.Neurophysiol.,* 94(6), 4387-4400.

Fox, K. (1992) 'A critical period for experience-dependent synaptic plasticity in rat barrel cortex', *J.Neurosci.,* 12, 1826-1838.

Fox, K. (2002) 'Anatomical pathways and molecular mechanisms for plasticity in the barrel cortex', *Neuroscience,* 111(4), 799-814.

Fox, K. (2008) *Barrel Cortex,* Cambridge, UK: Cambridge University Press.

Fox, K. (2009) 'Experience-dependent plasticity mechanisms for neural rehabilitation in somatosensory cortex', *Philos Trans R Soc Lond B Biol Sci,* 364(1515), 369-81.

Fox, K., Wallace, H. and Glazewski, S. (2002) 'Is there a thalamic component to experience dependent cortical plasticity?', *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences,* 357(1428), 1709-1715.

Fox, K. and Wong, R. O. (2005) 'A comparison of experience-dependent plasticity in the visual and somatosensory systems', *Neuron,* 48(3), 465-477.

Fredj, N. B. and Burrone, J. (2009) 'A resting pool of vesicles is responsible for spontaneous vesicle fusion at the synapse', *Nat Neurosci,* 12(6), 751-8.

Froemke, R. C. and Martins, A. R. (2011) 'Spectrotemporal dynamics of auditory cortical synaptic receptive field plasticity', *Hear Res,* 279(1-2), 149-61.

Froemke, R. C., Merzenich, M. M. and Schreiner, C. E. (2007) 'A synaptic memory trace for cortical receptive field plasticity', *Nature,* 450(7168), 425-429.

Galarreta, M. and Hestrin, S. (1998) 'Frequency-dependent synaptic depression and the balance of excitation and inhibition in the neocortex', *Nature Neuroscience,* 1(7), 587-594.

Galarreta, M. and Hestrin, S. (1999) 'A network of fast-spiking cells in the neocortex connected by electrical synapses', *Nature,* 402(6757), 72-75.

Galarreta, M. and Hestrin, S. (2002) 'Electrical and chemical synapses among parvalbumin fast-spiking GABAergic interneurons in adult mouse neocortex', *Proc.Natl.Acad.Sci.U.S.A,* 99(19), 12438-12443.

Gambino, F. and Holtmaat, A. (2012) 'Spike-timing-dependent potentiation of sensory surround in the somatosensory cortex is facilitated by deprivation-mediated disinhibition', *Neuron,* 75(3), 490-502.

Garraghty, P. E., LaChica, E. A. and Kaas, J. H. (1991) 'Injury-induced reorganization of somatosensory cortex is accompanied by reductions in GABA staining', *Somatosens Mot Res,* 8(4), 347-54.

Gentet, L. J. (2012) 'Functional diversity of supragranular GABAergic neurons in the barrel cortex', *Front Neural Circuits,* 6, 52.

Gibson, J. R., Beierlein, M. and Connors, B. W. (1999) 'Two networks of electrically coupled inhibitory neurons in neocortex', *Nature,* 402, 75-79.

Glazewski, S. and Fox, K. (1996) 'Time course of experience-dependent synaptic potentiation and depression in barrel cortex of adolescent rats', *J.Neurophysiol.,* 75(4), 1714-1729.

Glazewski, S., Herman, C., McKenna, M., Chapman, P. F. and Fox, K. (1998a) 'Long-term potentiation in vivo in layers II/III of rat barrel cortex', *Neuropharmacology,* 37(4-5), 581-92.

Glazewski, S., McKenna, M., Jacquin, M. and Fox, K. (1998b) 'Experience-dependent depression of vibrissae responses in adolescent rat barrel cortex', *Eur.J.Neurosci.,* 10, 2107-2116.

Goel, A. and Lee, H. K. (2007) 'Persistence of experience-induced homeostatic synaptic plasticity through adulthood in superficial layers of mouse visual cortex', *J Neurosci,* 27(25), 6692-700.

Goldberg, E. M., Jeong, H. Y., Kruglikov, I., Tremblay, R., Lazarenko, R. M. and Rudy, B. (2011) 'Rapid developmental maturation of neocortical FS cell intrinsic excitability', *Cereb Cortex,* 21(3), 666-82.

Goldberg, E. M., Watanabe, S., Chang, S. Y., Joho, R. H., Huang, Z. J., Leonard, C. S. and Rudy, B. (2005) 'Specific functions of synaptically localized potassium channels in synaptic transmission at the neocortical GABAergic fast-spiking cell synapse', *J Neurosci,* 25(21), 5230-5.

Groemer, T. W. and Klingauf, J. (2007) 'Synaptic vesicles recycling spontaneously and during activity belong to the same vesicle pool', *Nat Neurosci,* 10(2), 145-7.

Gupta, A., Wang, Y. and Markram, H. (2000) 'Organizing principles for a diversity of GABAergic interneurons and synapses in the neocortex', *Science,* 287(5451), 273-278.

Haider, B., Hausser, M. and Carandini, M. (2013) 'Inhibition dominates sensory responses in the awake cortex', *Nature,* 493(7430), 97-100.

Hardingham, N. and Fox, K. (2006) 'The role of nitric oxide and GluR1 in presynaptic and postsynaptic components of neocortical potentiation', *J.Neurosci.,* 26(28), 7395-7404.

Hardingham, N., Glazewski, S., Pakhotin, P., Mizuno, K., Chapman, P. F., Giese, K. P. and Fox, K. (2003) 'Neocortical long-term potentiation and experience-dependent synaptic plasticity require alpha-calcium/calmodulin-dependent protein kinase II autophosphorylation', *J.Neurosci.,* 23(11), 4428-4436.

Hendry, S. H. and Jones, E. G. (1988) 'Activity-dependent regulation of GABA expression in the visual cortex of adult monkeys', *Neuron,* 1(8), 701-712.

Hendry, S. H. C. and Jones, E. G. (1986) 'Reduction in number of immunostained GABAergic neurones in deprived-eye dominance columns of monkey area 17', *Nature,* 320, 750-753.

Hensch, T. K. (2005) 'Critical period plasticity in local cortical circuits', *Nat.Rev.Neurosci.,* 6(11), 877-888.

Hensch, T. K. and Fagiolini, M. (2005) 'Excitatory-inhibitory balance and critical period plasticity in developing visual cortex', *Prog.Brain Res.,* 147, 115-124.

Hensch, T. K., Fagiolini, M., Mataga, N., Stryker, M. P., Baekkeskov, S. and Kash, S. F. (1998) 'Local GABA circuit control of experience-dependent plasticity in developing visual cortex', *Science,* 282(5393), 1504-1508.

Hestrin, S. and Galarreta, M. (2005) 'Electrical synapses define networks of neocortical GABAergic neurons', *Trends Neurosci.,* 28(6), 304-309.

Hickmott, P. W. (2005) 'Changes in intrinsic properties of pyramidal neurons in adult rat S1 during cortical reorganization', *J.Neurophysiol.,* 94(1), 501-511.

Hickmott, P. W. and Ethell, I. M. (2006) 'Dendritic plasticity in the adult neocortex', *Neuroscientist,* 12(1), 16-28.

Hickmott, P. W. and Steen, P. A. (2005) 'Large-scale changes in dendritic structure during reorganization of adult somatosensory cortex', *Nat.Neurosci.,* 8(2), 140-142.

Hofer, S. B., Mrsic-Flogel, T. D., Bonhoeffer, T. and Hubener, M. (2006) 'Prior experience enhances plasticity in adult visual cortex', *Nat.Neurosci.,* 9(1), 127-132.

Hofer, S. B., Mrsic-Flogel, T. D., Bonhoeffer, T. and Hubener, M. (2009) 'Experience leaves a lasting structural trace in cortical circuits', *Nature,* 457(7227), 313-317.

House, D. R., Elstrott, J., Koh, E., Chung, J. and Feldman, D. E. (2011) 'Parallel regulation of feedforward inhibition and excitation during whisker map plasticity', *Neuron,* 72(5), 819-31.

Hua, Y., Sinha, R., Martineau, M., Kahms, M. and Klingauf, J. (2010) 'A common origin of synaptic vesicles undergoing evoked and spontaneous fusion', *Nat Neurosci,* 13(12), 1451-3.

Hubener, M. and Bonhoeffer, T. (2010) 'Searching for engrams', *Neuron,* 67(3), 363-71.

Inagaki, T., Begum, T., Reza, F., Horibe, S., Inaba, M., Yoshimura, Y. and Komatsu, Y. (2008) 'Brain-derived neurotrophic factor-mediated retrograde signaling required for the induction of long-term potentiation at inhibitory synapses of visual cortical pyramidal neurons', *Neurosci Res,* 61(2), 192-200.

Jacob, V., Petreanu, L., Wright, N., Svoboda, K. and Fox, K. (2012) 'Regular spiking and intrinsic bursting pyramidal cells show orthogonal forms of experience-dependent plasticity in layer V of barrel cortex', *Neuron,* 73(2), 391-404.

Jiang, B., Huang, S., de Pasquale, R., Millman, D., Song, L., Lee, H. K., Tsumoto, T. and Kirkwood, A. (2010) 'The maturation of GABAergic transmission in visual cortex requires endocannabinoid-mediated LTD of inhibitory inputs during a critical period', *Neuron,* 66(2), 248-59.

Jiao, Y., Zhang, C., Yanagawa, Y. and Sun, Q. Q. (2006) 'Major effects of sensory experiences on the neocortical inhibitory circuits', *J.Neurosci.,* 26(34), 8691-8701.

Jones, E. G. (1993) 'GABAergic neurons and their role in cortical plasticity in primates', *Cereb.Cortex.,* 3(5), 361-372.

Jones, E. G. and Pons, T. P. (1998) 'Thalamic and brainstem contributions to large-scale plasticity of primate somatosensory cortex', *Science,* 282, 1121-1125.

Kaas, J. H. (1987) 'The organization of cortex in mammals: Implications for theories of brain function', *Annu.Rev.Psychol.,* 38, 124-151.

Kalisman, N., Silberberg, G. and Markram, H. (2005) 'The neocortical microcircuit as a tabula rasa', *Proc.Natl.Acad.Sci.U.S.A.,* 102(3), 880-885.

Kawaguchi, Y. (1995) 'Physiological subgroups of nonpyramidal cells with specific morphological characteristics in layer II/III of rat frontal cortex', *J.Neurosci.,* 15, 2638-2655.

Kawaguchi, Y. and Kubota, Y. (1993) 'Correlation of physiological subgroupings of nonpyramidal cells with parvalbumin- and calbindinD28k-immunoreactive neurons in layer V of rat frontal cortex', *J.Neurophysiol.,* 70(1), 387-396.

Kawaguchi, Y. and Kubota, Y. (1997) 'GABAergic cell types and their synaptic connections in rat frontal cortex', *Cerebral Cortex,* 7, 476-486.

Kawaguchi, Y. and Shindou, T. (1998) 'Noradrenergic excitation and inhibition of GABAergic cell types in rat frontal cortex', *J.Neurosci.,* 18(17), 6963-6976.

Keck, T., Mrsic-Flogel, T. D., Vaz, A. M., Eysel, U. T., Bonhoeffer, T. and Hubener, M. (2008) 'Massive restructuring of neuronal circuits during functional reorganization of adult visual cortex', *Nat.Neurosci.,* 11(10), 1162-1167.

Keck, T., Scheuss, V., Jacobsen, R. I., Wierenga, C. J., Eysel, U. T., Bonhoeffer, T. and Hubener, M. (2011) 'Loss of sensory input causes rapid structural changes of inhibitory neurons in adult mouse visual cortex', *Neuron,* 71(5), 869-82.

Kelly, M. K., Carvell, G. E., Kodger, J. M. and Simons, D. J. (1999) 'Sensory loss by selected whisker removal produces immediate disinhibition in the somatosensory cortex of behaving rats', *J.Neurosci.,* 19, 9117-9125.

Kilgard, M. P. and Merzenich, M. M. (1998) 'Cortical map reorganization enabled by nucleus basalis activity', *Science,* 279(5357), 1714-1718.

Kilman, V., van Rossum, M. C. and Turrigiano, G. G. (2002) 'Activity deprivation reduces miniature IPSC amplitude by decreasing the number of postsynaptic GABA(A) receptors clustered at neocortical synapses', *J Neurosci,* 22(4), 1328-37.

Knott, G. W., Quairiaux, C., Genoud, C. and Welker, E. (2002) 'Formation of dendritic spines with GABAergic synapses induced by whisker stimulation in adult mice', *Neuron,* 34(2), 265-273.

Kobayashi, M., Hamada, T., Kogo, M., Yanagawa, Y., Obata, K. and Kang, Y. (2008) 'Developmental profile of GABAA-mediated synaptic transmission in pyramidal cells of the somatosensory cortex', *Eur J Neurosci,* 28(5), 849-61.

Kossut, M. and Hand, P. (1984) 'Early development of changes in cortical representation of C3 vibrissa following neonatal denervation of surrounding vibrissa receptors: a 2-deoxyglucose study in the rat', *Neurosci Lett,* 46(1), 7-12.

Kossut, M., Hand, P. J., Greenberg, J. and Hand, C. L. (1988) 'Single vibrissal cortical column in SI cortex of rat and its alterations in neonatal and adult vibrissa-deafferented animals: a quantitative 2DG study', *J.Neurophysiol.,* 60(2), 829-852.

Kubota, Y., Hattori, R. and Yui, Y. (1994) 'Three distinct subpopulations of GABAergic neurons in rat frontal agranular cortex', *Brain Res.,* 649(1-2), 159-173.

Kullmann, D. M. and Lamsa, K. P. (2011) 'LTP and LTD in cortical GABAergic interneurons: emerging rules and roles', *Neuropharmacology,* 60(5), 712-9.

Kullmann, D. M., Moreau, A. W., Bakiri, Y. and Nicholson, E. (2012) 'Plasticity of inhibition', *Neuron,* 75(6), 951-62.

Lamsa, K., Heeroma, J. H. and Kullmann, D. M. (2005) 'Hebbian LTP in feed-forward inhibitory interneurons and the temporal fidelity of input discrimination', *Nat Neurosci,* 8(7), 916-924.

Lamsa, K., Irvine, E. E., Giese, K. P. and Kullmann, D. M. (2007) 'NMDA receptor-dependent long-term potentiation in mouse hippocampal interneurons shows a unique dependence on Ca(2+)/calmodulin-dependent kinases', *J.Physiol,* 584(Pt 3), 885-894.

Lamsa, K. P., Kullmann, D. M. and Woodin, M. A. (2010) 'Spike-timing dependent plasticity in inhibitory circuits', *Front Synaptic Neurosci,* 2, 8.

Land, P. W. and Simons, D. J. (1985) 'Metabolic activity in SmI cortical barrels of adult rats is dependent on patterned sensory stimulation of the mystacial vibrissae', *Brain Res.,* 341(1), 189-194.

Lebedev, M. A., Mirabella, G., Erchova, I. and Diamond, M. E. (2000) 'Experience-dependent plasticity of rat barrel cortex: redistribution of activity across barrel-columns', *Cerebral Cortex,* 10(1), 23-31.

Lee, W. C., Chen, J. L., Huang, H., Leslie, J. H., Amitai, Y., So, P. T. and Nedivi, E. (2008) 'A dynamic zone defines interneuron remodeling in the adult neocortex', *Proc.Natl.Acad.Sci.U.S.A,* 105(50), 19968-19973.

Lee, W. C., Huang, H., Feng, G., Sanes, J. R., Brown, E. N., So, P. T. and Nedivi, E. (2006) 'Dynamic remodeling of dendritic arbors in GABAergic interneurons of adult visual cortex', *PLoS.Biol.,* 4(2), e29.

Levelt, C. N. and Hubener, M. (2012) 'Critical-period plasticity in the visual cortex', *Annu Rev Neurosci,* 35, 309-30.

Li, X., Glazewski, S., Lin, X., Elde, R. and Fox, K. (1995) 'Effect of vibrissae deprivation on follicle innervation, neuropeptide synthesis in the trigeminal ganglion, and S1 barrel cortex plasticity', *J.Comp.Neurol.,* 357(3), 465-481.

Liang, K. Y. and Zeger, S. L. (1986) 'Longitudinal data-analysis using generalized linear-models', *Biometrika,* 73(1), 13-22.

Liu, G. S. and Tsien, R. W. (1995) 'Properties of synaptic transmission at single hippocampal synaptic boutons', *Nature,* 375(6530), 404-408.

Lovett-Barron, M., Turi, G. F., Kaifosh, P., Lee, P. H., Bolze, F., Sun, X. H., Nicoud, J. F., Zemelman, B. V., Sternson, S. M. and Losonczy, A. (2012) 'Regulation of neuronal input transformations by tunable dendritic inhibition', *Nat Neurosci,* 15(3), 423-30, S1-3.

Lu, J. T., Li, C. Y., Zhao, J. P., Poo, M. M. and Zhang, X. H. (2007) 'Spike-timing-dependent plasticity of neocortical excitatory synapses on inhibitory interneurons depends on target cell type', *J.Neurosci.,* 27(36), 9711-9720.

Lubke, J., Egger, V., Sakmann, B. and Feldmeyer, D. (2000) 'Columnar organization of dendrites and axons of single and synaptically coupled excitatory spiny neurons in layer 4 of the rat barrel cortex', *J.Neurosci.,* 20(14), 5300-5311.

Ma, Y., Hu, H. and Agmon, A. (2012) 'Short-term plasticity of unitary inhibitory-to-inhibitory synapses depends on the presynaptic interneuron subtype', *J Neurosci,* 32(3), 983-8.

Maffei, A., Nataraj, K., Nelson, S. B. and Turrigiano, G. G. (2006) 'Potentiation of cortical inhibition by visual deprivation', *Nature,* 443(7107), 81-84.

Maffei, A., Nelson, S. B. and Turrigiano, G. G. (2004) 'Selective reconfiguration of layer 4 visual cortical circuitry by visual deprivation', *Nat.Neurosci.,* 7(12), 1353-1359.

Maffei, A. and Turrigiano, G. G. (2008) 'Multiple modes of network homeostasis in visual cortical layer 2/3', *J.Neurosci.,* 28(17), 4377-4384.

Maravall, M., Stern, E. A. and Svoboda, K. (2004) 'Development of intrinsic properties and excitability of layer 2/3 pyramidal neurons during a critical period for sensory maps in rat barrel cortex', *J.Neurophysiol.,* 92(1), 144-156.

Margolis, D. J., Lutcke, H., Schulz, K., Haiss, F., Weber, B., Kugler, S., Hasan, M. T. and Helmchen, F. (2012) 'Reorganization of cortical population activity imaged throughout long-term sensory deprivation', *Nat Neurosci,* 15(11), 1539-46.

Marik, S. A., Yamahachi, H., McManus, J. N., Szabo, G. and Gilbert, C. D. (2010) 'Axonal dynamics of excitatory and inhibitory neurons in somatosensory cortex', *PLoS.Biol.,* 8(6), e1000395.

Markram, H., Gerstner, W. and Sjostrom, P. J. (2011) 'A history of spike-timing-dependent plasticity', *Front Synaptic Neurosci,* 3, 4.

Markram, H., Lubke, J., Frotscher, M. and Sakmann, B. (1997) 'Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs', *Science,* 275(5297), 213-215.

Markram, H., Toledo-Rodriguez, M., Wang, Y., Gupta, A., Silberberg, G. and Wu, C. Z. (2004) 'Interneurons of the neocortical inhibitory system', *Nat.Rev.Neurosci.,* 5(10), 793-807.

Markram, H. and Tsodyks, M. (1996) 'Redistribution of synaptic efficacy between neocortical pyramidal cells', *Nature,* 382, 807-810.

Maya-Vetencourt, J. F., Sale, A., Viegi, A., Baroncelli, L., De Pasquale, R., O'Leary, O. F., Castren, E. and Maffei, L. (2008) 'The antidepressant fluoxetine restores plasticity in the adult visual cortex', *Science,* 320(5874), 385-388.

McCormick, D. A., Connors, B. W., Lighthall, J. W. and Prince, D. A. (1985) 'Comparative electrophysiology of pyramidal and sparsely spiny stellate neurons of the neocortex', *J.Neurophysiol.,* 54(4), 782-806.

Merzenich, M. M., Kaas, J. H., Wall, J., Nelson, R. J., Sur, M. and Felleman, D. (1983a) 'Progression of changes following median nerve section in the cortical representation of the hand in areas 3b and 1 in adult owl and squirrel monkeys', *Neuroscience,* 10, 639-665.

Merzenich, M. M., Kaas, J. H., Wall, J., Nelson, R. J., Sur, M. and Felleman, D. (1983b) 'Topographic reorganisation of somatosensory cortical areas 3b and 1 in adult monkeys following restricted deafferentation', *Neuroscience,* 8, 33-55.

Meyer, H. S., Schwarz, D., Wimmer, V. C., Schmitt, A. C., Kerr, J. N., Sakmann, B. and Helmstaedter, M. (2011) 'Inhibitory interneurons in a cortical column form hot zones of inhibition in layers 2 and 5A', *Proc Natl Acad Sci U S A,* 108(40), 16807-12.

Micheva, K. D. and Beaulieu, C. (1996) 'Quantitative aspects of synaptogenesis in the rat barrel field cortex with special reference to GABA circuitry', *J Comp Neurol,* 373(3), 340-54.

Micheva, K. D. and Beaulieu, C. (1997) 'Development and plasticity of the inhibitory neocortical circuitry with an emphasis on the rodent barrel field cortex: a review', *Can.J.Physiol Pharmacol.,* 75(5), 470-478.

Mitchell, S. J. and Silver, R. A. (2003) 'Shunting inhibition modulates neuronal gain during synaptic excitation', *Neuron,* 38(3), 433-445.

Moore, C. I., Nelson, S. B. and Sur, M. (1999) 'Dynamics of neuronal processing in rat somatosensory cortex', *Trends Neurosci.,* 22(11), 513-520.

Morales, B., Choi, S. Y. and Kirkwood, A. (2002) 'Dark rearing alters the development of GABAergic transmission in visual cortex', *J Neurosci,* 22(18), 8084-90.

Morishita, H., Miwa, J. M., Heintz, N. and Hensch, T. K. (2010) 'Lynx1, a cholinergic brake, limits plasticity in adult visual cortex', *Science,* 330(6008), 1238-1240.

Mountcastle, V. B. (1997) 'The columnar organization of the cortex', *Brain,* 120, 701-722.

Mountcastle, V. B., Talbot, W. H., Sakata, H. and Hyvarinen, J. (1969) 'Cortical neuronal mechanisms in flutter-vibration studied in unanesthetized monkeys. Neuronal periodicity and frequency discrimination', *J Neurophysiol,* 32(3), 452-84.

Nudo, R. J. (2006) 'Mechanisms for recovery of motor function following cortical damage', *Current Opinions in Neurobiology,* 16(6), 638-644.

Nunez, A., Dominguez, S., Buno, W. and Fernandez de Sevilla, D. (2012) 'Cholinergic-mediated response enhancement in barrel cortex layer V pyramidal neurons', *J Neurophysiol,* 108(6), 1656-68.

Oberlaender, M., Ramirez, A. and Bruno, R. M. (2012) 'Sensory experience restructures thalamocortical axons during adulthood', *Neuron,* 74(4), 648-55.

Oren, I., Nissen, W., Kullmann, D. M., Somogyi, P. and Lamsa, K. P. (2009) 'Role of ionotropic glutamate receptors in long-term potentiation in rat hippocampal CA1 oriens-lacunosum moleculare interneurons', *J Neurosci,* 29(4), 939-50.

Packer, A. M. and Yuste, R. (2011) 'Dense, unspecific connectivity of neocortical parvalbumin-positive interneurons: a canonical microcircuit for inhibition?', *J Neurosci,* 31(37), 13260-71.

Palop, J. J., Chin, J. and Mucke, L. (2006) 'A network dysfunction perspective on neurodegenerative diseases', *Nature,* 443(7113), 768-773.

Peters, A. and Jones, E. G. (1984) 'Classification of Cortical Neurons' in Peters, A. and Jones, E. G., eds., *Cerebral Cortex. Vol 1 Cellular Components of the Cerebral Cortex*, New York: Plenum Press, 107-121.

Petersen, C. C. (2007) 'The functional organization of the barrel cortex', *Neuron,* 56(2), 339-355.

Petersen, C. C. and Sakmann, B. (2001) 'Functionally independent columns of rat somatosensory barrel cortex revealed with voltage-sensitive dye imaging', *J.Neurosci.,* 21(21), 8435-8446.

Picciotto, M. R., Higley, M. J. and Mineur, Y. S. (2012) 'Acetylcholine as a neuromodulator: cholinergic signaling shapes nervous system function and behavior', *Neuron,* 76(1), 116-29.

Pierret, T., Lavallee, P. and Deschenes, M. (2000) 'Parallel streams for the relay of vibrissal information through thalamic barreloids', *Journal of Neuroscience,* 20(19), 7455-7462.

Pizzorusso, T., Medini, P., Berardi, N., Chierzi, S., Fawcett, J. W. and Maffei, L. (2002) 'Reactivation of ocular dominance plasticity in the adult visual cortex', *Science,* 298(5596), 1248-1251.

Pizzorusso, T., Medini, P., Landi, S., Baldini, S., Berardi, N. and Maffei, L. (2006) 'Structural and functional recovery from early monocular deprivation in adult rats', *Proc Natl Acad Sci U S A,* 103(22), 8517-22.

Povysheva, N. V., Gonzalez-Burgos, G., Zaitsev, A. V., Kroner, S., Barrionuevo, G., Lewis, D. A. and Krimer, L. S. (2006) 'Properties of excitatory synaptic responses in fast-spiking interneurons and pyramidal cells from monkey and rat prefrontal cortex', *Cereb Cortex,* 16(4), 541-52.

Recanzone, G. H., Merzenich, M. M. and Jenkins, W. M. (1992a) 'Frequency discrimination training engaging a restricted skin surface results in an emergence of a cutaneous response zone in cortical area 3a', *J.Neurophysiol.,* 67(5), 1057-1070.

Recanzone, G. H., Merzenich, M. M., Jenkins, W. M., Grajski, K. A. and Dinse, H. R. (1992b) 'Topographic reorganization of the hand representation in cortical area 3b owl monkeys trained in a frequency-discrimination task', *J.Neurophysiol.,* 67(5), 1031-1056.

Reyes, A., Lujan, R., Rozov, A., Burnashev, N., Somogyi, P. and Sakmann, B. (1998) 'Target-cell-specific facilitation and depression in neocortical circuits', *Nat.Neurosci.,* 1, 279-285.

Reyes, A. and Sakmann, B. (1999) 'Developmental switch in the short-term modification of unitary EPSPs evoked in layer 2/3 and layer 5 pyramidal neurons of rat neocortex', *J.Neurosci.,* 19(10), 3827-3835.

Rizzoli, S. O. and Betz, W. J. (2005) 'Synaptic vesicle pools', *Nat.Rev.Neurosci.,* 6(1), 57-69.

Rudy, B. (1999) 'Molecular diversity of ion channels and cell function', *Ann N Y Acad Sci,* 868, 1-12.

Rudy, B., Fishell, G., Lee, S. and Hjerling-Leffler, J. (2011) 'Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons', *Dev Neurobiol,* 71(1), 45-61.

Rudy, B. and McBain, C. J. (2001) 'Kv3 channels: voltage-gated K+ channels designed for high-frequency repetitive firing', *Trends Neurosci,* 24(9), 517-26.

Sachdev, R. N., Lu, S. M., Wiley, R. G. and Ebner, F. F. (1998) 'Role of the basal forebrain cholinergic projection in somatosensory cortical plasticity', *J Neurophysiol,* 79(6), 3216-28.

Salin, P. A. and Prince, D. A. (1996) 'Spontaneous GABAA receptor-mediated inhibitory currents in adult rat somatosensory cortex', *J.Neurophysiol.,* 75(4), 1573-1588.

Sara, Y., Virmani, T., Deak, F., Liu, X. and Kavalali, E. T. (2005) 'An isolated pool of vesicles recycles at rest and drives spontaneous neurotransmission', *Neuron,* 45(4), 563-573.

Sarihi, A., Jiang, B., Komaki, A., Sohya, K., Yanagawa, Y. and Tsumoto, T. (2008) 'Metabotropic glutamate receptor type 5-dependent long-term potentiation of excitatory synapses on fast-spiking GABAergic neurons in mouse visual cortex', *J Neurosci,* 28(5), 1224-35.

Simkus, C. R. and Stricker, C. (2002) 'Properties of mEPSCs recorded in layer II neurones of rat barrel cortex', *J.Physiol.(Lond.),* 545(Pt 2), 509-520.

Simon, A., Olah, S., Molnar, G., Szabadics, J. and Tamas, G. (2005) 'Gap-junctional coupling between neurogliaform cells and various interneuron types in the neocortex', *J Neurosci,* 25(27), 6278-85.

Simons, D. J. and Land, P. W. (1987) 'Early experience of tactile stimulation influences organization of somatic sensory cortex', *Nature,* 326(6114), 694-697.

Somogyi, P., Freund, T. F. and Cowey, A. (1982) 'The axo-axonic interneuron in the cerebral cortex of the rat, cat and monkey', *Neuroscience,* 7(11), 2577-607.

Somogyi, P. and Klausberger, T. (2005) 'Defined types of cortical interneurone structure space and spike timing in the hippocampus', *J.Physiol,* 562(Pt 1), 9-26.

Song, C., Detert, J. A., Sehgal, M. and Moyer, J. R., Jr. (2012) 'Trace fear conditioning enhances synaptic and intrinsic plasticity in rat hippocampus', *J Neurophysiol,* 107(12), 3397-408.

Southwell, D. G., Froemke, R. C., varez-Buylla, A., Stryker, M. P. and Gandhi, S. P. (2010) 'Cortical plasticity induced by inhibitory neuron transplantation', *Science,* 327(5969), 1145-1148.

Spruston, N., Jaffe, D. B., Williams, S. H. and Johnston, D. (1993) 'Voltage- and space-clamp errors associated with the measurement of electrotonically remote synaptic events', *J.Neurophysiol.,* 70(2), 781-802.

Stern, E. A., Maravall, M. and Svoboda, K. (2001) 'Rapid development and plasticity of layer 2/3 maps in rat barrel cortex in vivo', *Neuron,* 31(2), 305-315.

Strichartz, G. R. (1973) 'The inhibition of sodium currents in myelinated nerve by quaternary derivatives of lidocaine', *J Gen Physiol,* 62(1), 37-57.

Sun, Q. Q. (2009) 'Experience-dependent intrinsic plasticity in interneurons of barrel cortex layer IV', *J Neurophysiol,* 102(5), 2955-73.

Syken, J., GrandPre, T., Kanold, P. O. and Shatz, C. J. (2006) 'PirB restricts ocular-dominance plasticity in visual cortex', *Science,* 313(5794), 1795-1800.

Thomson, A. M. (1997) 'Activity-dependent properties of synaptic transmission at two classes of connections made by rat neocortical pyramidal axons in vitro', *J.Physiol.(Lond.),* 502(1), 131-147.

Thomson, A. M. and Deuchars, J. (1997) 'Synaptic interactions in neocortical local circuits: dual intracellular recordings in vitro', *Cerebral Cortex,* 7(6), 510-522.

Thomson, A. M. and Lamy, C. (2007) 'Functional maps of neocortical local circuitry', *Front Neurosci,* 1(1), 19-42.

Trachtenberg, J. T., Chen, B. E., Knott, G. W., Feng, G., Sanes, J. R., Welker, E. and Svoboda, K. (2002) 'Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex', *Nature,* 420(6917), 788-794.

Turrigiano, G. G. (1999) 'Homeostatic plasticity in neuronal networks: the more things change, the more they stay the same', *Trends Neurosci.,* 22(5), 221-227.

Turrigiano, G. G. (2008) 'The self-tuning neuron: synaptic scaling of excitatory synapses', *Cell,* 135(3), 422-435.

Turrigiano, G. G., Leslie, K. R., Desai, N. S., Rutherford, L. and Nelson, S. B. (1998) 'Activity-dependent scaling of quantal amplitude in neocortical neurons', *Nature,* 391, 892-896.

Van der Loos, H. and Woolsey, T. A. (1973) 'Somatosensory cortex: structural alterations following early injury to sense organs', *Science,* 179(4071), 395-8.

van Versendaal, D., Rajendran, R., Saiepour, M. H., Klooster, J., Smit-Rigter, L., Sommeijer, J. P., De Zeeuw, C. I., Hofer, S. B., Heimel, J. A. and Levelt, C. N. (2012) 'Elimination of inhibitory synapses is a major component of adult ocular dominance plasticity', *Neuron,* 74(2), 374-83.

Vorobyov, V., Kwok, J. C., Fawcett, J. W. and Sengpiel, F. (2013) 'Effects of digesting chondroitin sulfate proteoglycans on plasticity in cat primary visual cortex', *J Neurosci,* 33(1), 234-43.

Wallace, H. and Fox, K. (1999a) 'The effect of vibrissa deprivation pattern on the form of plasticity induced in rat barrel cortex', *Somatosens.Mot.Res.,* 16(2), 122-138.

Wallace, H. and Fox, K. (1999b) 'Local cortical interactions determine the form of cortical plasticity', *Journal of Neurobiology,* 41(1), 58-63.

Wallace, H., Glazewski, S., Liming, K. and Fox, K. (2001) 'The role of cortical activity in experience-dependent potentiation and depression of sensory responses in rat barrel cortex', *J.Neurosci.,* 21(11), 3881-3894.

Wehr, M. and Zador, A. M. (2003) 'Balanced inhibition underlies tuning and sharpens spike timing in auditory cortex', *Nature,* 426(6965), 442-446.

Welker, C. and Woolsey, T. A. (1974) 'Structure of layer IV in the somatosensory neocortex of the rat: description and comparison with the mouse', *J.Comp.Neurol.,* 158, 437-454.

Welker, W. I. (1964) 'Analysis of sniffing of the albino rat', *Behaviour,* 22 223-244.

Wheeler, D. B., Randall, A. and Tsien, R. W. (1996) 'Changes in action potential duration alter reliance of excitatory synaptic transmission on multiple types of Ca2+ channels in rat hippocampus', *J Neurosci,* 16(7), 2226-37.

Wilhelm, B. G., Groemer, T. W. and Rizzoli, S. O. (2010) 'The same synaptic vesicles drive active and spontaneous release', *Nat Neurosci,* 13(12), 1454-6.

Williams, S. R. and Mitchell, S. J. (2008) 'Direct measurement of somatic voltage clamp errors in central neurons', *Nat Neurosci,* 11(7), 790-8.

Wirth, C. and Luscher, H. R. (2004) 'Spatiotemporal evolution of excitation and inhibition in the rat barrel cortex investigated with multielectrode arrays', *J Neurophysiol.,* 91(4), 1635-1647.

Woodruff, A., Xu, Q., Anderson, S. A. and Yuste, R. (2009) 'Depolarizing effect of neocortical chandelier neurons', *Front Neural Circuits,* 3, 15.

Woolsey, T. A. and Van der Loos, H. (1970) 'The structural organization of layer IV in the somatosensory region (S I) of mouse cerebral cortex', *Brain Res.,* 17, 205-242.

Woolsey, T. A. and Wann, J. R. (1976) 'Areal changes in mouse cortical barrels following vibrissal damage at different postnatal ages', *J.Comp.Neurol.,* 170, 53-66.

Wright, A. K., Norrie, L. and Arbuthnott, G. W. (2000) 'Corticofugal axons from adjacent 'barrel' columns of rat somatosensory cortex: cortical and thalamic terminal patterns', *J Anat,* 196 (Pt 3), 379-90.

Yizhar, O., Fenno, L. E., Prigge, M., Schneider, F., Davidson, T. J., O'Shea, D. J., Sohal, V. S., Goshen, I., Finkelstein, J., Paz, J. T., Stehfest, K., Fudim, R., Ramakrishnan, C., Huguenard, J. R., Hegemann, P. and Deisseroth, K. (2011) 'Neocortical excitation/inhibition balance in information processing and social dysfunction', *Nature,* 477(7363), 171-178.

Yoshimura, Y., Ohmura, T. and Komatsu, Y. (2003) 'Two forms of synaptic plasticity with distinct dependence on age, experience, and NMDA receptor subtype in rat visual cortex', *Journal of Neuroscience,* 23(16), 6557-6566.

Yu, X., Chung, S., Chen, D. Y., Wang, S., Dodd, S. J., Walters, J. R., Isaac, J. T. and Koretsky, A. P. (2012) 'Thalamocortical inputs show post-critical-period plasticity', *Neuron,* 74(4), 731-42.

Yuste, R. (2005) 'Origin and classification of neocortical interneurons', *Neuron,* 48(4), 524-527.

Zeger, S. L. and Liang, K. Y. (1986) 'Longitudinal data analysis for discrete and continuous outcomes', *Biometrics,* 42(1), 121-130.

Zhang, W. and Linden, D. J. (2003) 'The other side of the engram: experience-driven changes in neuronal intrinsic excitability', *Nat.Rev.Neurosci.,* 4(11), 885-900.

Zhong, P. and Yan, Z. (2011) 'Differential regulation of the excitability of prefrontal cortical fast-spiking interneurons and pyramidal neurons by serotonin and fluoxetine', *PLoS One,* 6(2), e16970.

Zucker, R. S. and Regehr, W. G. (2002) 'Short-term synaptic plasticity', *Annu.Rev.Physiol.,* 64, 355-405.